BETACATENIN EXPRESSION IN COLORECTAL ADENOMA AND CARCINOMA

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CERTIFICATE

This is to certify that this dissertation entitled "BETACATENIN EXPRESSION IN COLORECTAL ADENOMA AND CARCINOMA" is the bonafide record of the research work done by Dr. T.ANNIE REXALIN PRADEEPA, submitted, in partial fulfilment of the regulations laid down by the Tamilnadu Dr.M.G.R. Medical University, Chennai, for M.D. (Pathology) Degree Examination to be held in May 2019.

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ACKNOWLEDGEMENT FROM GUIDE

This is to certify that Dr. T.ANNIE REXALIN PRADEEPA, Post Graduate Student (2016-2019) in the department of Pathology, GOVERNMENT KILPAUK MEDICAL COLLEGE AND HOSPITAL, Chennai - 600 010, has done this dissertation on "BETACATENIN EXPRESSION IN COLORECTAL ADENOMA AND CARCINOMA" under my guidance and supervision at Government Kilpauk Medical College, Chennai, in partial fulfilment of the regulations laid down by the Tamilnadu Dr.M.G.R. Medical University, Chennai, for M.D. (Pathology) Degree Examination to be held in May 2019.

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DECLARATION

I, Dr. T.ANNIE REXALIN PRADEEPA, declare that I carried out this work on "BETACATENIN EXPRESSION IN COLORECTAL ADENOMA AND CARCINOMA" at Department of Pathology, Government Kilpauk Medical College Hospital. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, and diploma to any other university.

This is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of the rules and regulation for the M. D. Degree examination in Pathology.

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Date :

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No words are enough to express my pride and deep sense of reverence I have for my husband, parents, in laws ,sisters and friends. Above all I remember the master of ceremonies, the powerful God, The Almighty who gave courage, enthusiasm and vigour to accomplish this work.

INSTITUTIONAL ETHICS COMMITTEE
GOVT. KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Protocol ID. No. 02/2017 Meeting held on 14.11.2017

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval **"BETA CATENIN EXPRESSION IN COLORECTAL ADENOMA AND CARCINOMA"** submitted by Dr.T.ANNIE RELAXIN PRADEEPA, Post Graduate in Pathology, Govt. Kilpauk Medical College, Chennai-10.

The Proposal is **APPROVED.**

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

14.11.2017

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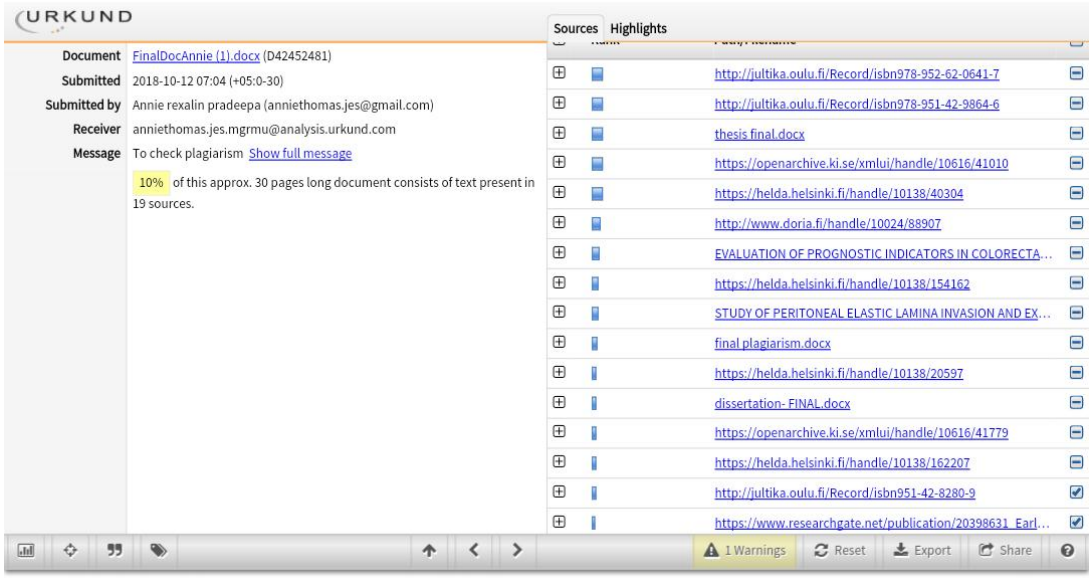
ME 1 Sec> Ethical Committee



ABBREVIATIONS

VEGF Vascular Endothelial Growth Factor FAP Familial Adenomatous Polyposis APC Adenomatous Polyposis Coli HNPCC Hereditary Non-Polyposis Colon Cancer MSI Microsatellite In-stability MMR Mismatch Repair WHO World Health Organization AJCC American Joint Committee on Colon Cancer CEA Carcino EmbryonicAntigen CK CytoKeratin GIST Gastrointestinal Stromal Tumour FFPE Formalin Fixed Paraffin Embedded NSAIDs Non-Steroidal Anti-Inflammatory Drugs IEL IntraepithelialLymphocytes COX Cyclooxygenase

ABBREVIATIONS

VEGF	Vascular Endothelial Growth Factor
FAP	Familial Adenomatous Polyposis
APC	Adenomatous Polyposis Coli
HNPCC	Hereditary Non-Polyposis Colon Cancer
MSI	Microsatellite In-stability
MMR	Mismatch Repair
WHO	World Health Organization
AJCC	American Joint Committee on Colon Cancer
CEA	Carcino EmbryonicAntigen
CK	CytoKeratin
GIST	Gastrointestinal Stromal Tumour
FFPE	Formalin Fixed Paraffin Embedded
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
IEL	IntraepithelialLymphocytes
COX	Cyclooxygenase

CONTENTS

ABSTRACT

INTRODUCTION

Adenocarcinoma of colon is the most common tumor of gastrointestinal tract. Malignant epithelial tumors of colon and rectum accounts for 85% of all cancers worldwide.[1,2] Colorectal cancer is a disease of late middle age and elderly individuals with a peak incidence at 60-70 with a male preponderance. It is the third most common cancer in males and second most common cancer in females. [1,3] .Classic adenoma-carcinoma sequence constitutes for about 80% Cases[4].The prognosis depends mainly on the stage of the disease.

Approximately 30% cases with colorectal cancer have metastasis at the time of first presentation . β –catenin/TCF pathway deregulation play a central role in colorectal cancers. Under physiological condition large fraction of β catenin is bound to cell membrane with E cadherin which is cell to cell adhesion protein and through α catenin to actin cytoskeleton, free unphosphorylated βcatenin translocate to nucleus. unphosphorylated βcatenin which binds to transcriptional factors TCF /LEF and activates transcription of specific target gene such as c-myc,cyclin D1,VEGF,MMP7 which leads to cell proliferation and carcinogenesis[5].APC /β –catenin/TCF pathway deregulation play a central role in colorectal Cancers.

AIMS & OBJECTIVES

This study aims,

To assess the expression of beta catenin in colorectal adenoma and carcinoma at a tertiary care hospital –Govt. Kilpauk Medical College, Chennai.

OBJECTIVES:

To find the rate of expression of beta catenin in colorectal adenoma and Carcinoma.

To correlate it with clinico pathological variables (age, sex, clinical features, gross and microscopic findings).

MATERIALS AND METHODS

50 resected specimens of colorectal carcinoma from January 2016 to May 2018 were studied. immunohistochemistry was done using beta catenin.

RESULTS:

Among 50 cases studied at Kilpauk medical college from 2016 - 2018,mean age of presentation in colorectal adenoma is 57.7 years and colorectal carcinoma is 48.8 years.

- Colorectal adenoma showed female preponderance, colorectal carcinoma showed male preponderance.
- Both adenoma and carcinoma showed left sided preponderance.
- Well differentiated Infiltrating adenocarcinoma being the predominant histopathological subtype.
- Immunohistochemically analysis was performed to assess the expression of beta catenin in colorectal adenoma and carcinoma.
- Significant association is present in membranous and cytoplasmic expression of beta catenin, which is higher in carcinoma when compared with adenoma.
- Out of 50 cases only 3 cases of carcinoma showed nuclear positivity.
- This study throws light on the need for targeted therapy against beta catenin which is overexpressed in carcinomas compared with adenoma.

KEY WORDS:

Colorectal adenoma,carcinoma, beta catenin,immunohistochemistry.

REVIEW OF LITERATURE

INTRODUCTION

Colorectal carcinoma is one among the common malignancies of the gastrointestinal tract with a peak age of occurrence is 60-70 years. The classic adenoma-carcinoma sequence is seen in about 80% cases. About 30%patients diagnosed as colorectal carcinoma had already regional or distant metastasis at the time of presentation.

EPIDEMIOLOGY:

Colorectal cancer is one of the most important public health problem and an important cause of morbidity and mortality all over the world[6]. There is a worldwide difference in global distribution of colorectal cancer. Countries with high risk include Australia, New Zealand, Canada and those with low risk include China, India, Africa, South America[7].

Global age standardized rates of colorectal carcinoma incidence are higher in men than in women (19.1/1,00,000 in men and 14.4/1,00,000 in women)[8.]

Of all the cases reported worldwide, incidence in developed world accounts for about 63% of cases[9]. Incidence rates varies up to 10-fold between countries with the highest rates and lowest rate[10,11]. Most colorectal cancers arise from benign adenomatous polyps lining the wall of the bowel[12].

Development of colorectal cancer is a multistep process[13].

INCIDENCE IN INDIA:

In India, the yearly incidence rates for colon and rectal cancer in men are 4.4 and 4.1/100000 respectively. Incidence in women is 3.9/100000.Colon cancer ranks eighth and rectal cancer ranks ninth among men. In women, rectal cancer does not fit in top ten, whereas colon cancer ranks ninth[14].

MORTALITY:

Colorectal cancer is the fourth most common cause of death from cancer globally[15]. Survival is very much dependent on stage of the disease at diagnosis and varies from 90% five years survival for cancers detected at the earlier stage, 70% in patients with regional lymph node metastasis and 10% in patients who present with distant metastasis[16,17].

ETIOLOGY AND RISK FACTORS:

The risk factors are classified as genetic, environmental, life style related factors.

GENETIC FACTORS:

Colorectal carcinomas may be associated with colonic polyposis.

Colonic polyposis syndrome includes:

- Familial adenomatous polyposis and its variants like Turcot syndrome, Gardner syndrome and attenuated FAP
- Hereditary non- polyposis colon cancer or Lynch syndrome comprises non-polyposis category.

- Familial adenomatous polyposis is characterized by multiple colonic adenomatous polyps appearing in childhood which later transformed to malignancy at an average age of 45 years and it is due to genetic mutation in APC gene on chromosome 5[18].
- Turcot syndrome is a variant of Familial adenomatous polyposis, presents with multiple colorectal adenomas and primary neuroepithelial brain tumors.
- Germline mutations of mismatch repair gene is demonstrated.

HEREDITARY NON-POLYPOSIS COLON CANCER (LYNCH SYNDROME):

It is an autosomal dominant condition. Defects in any one of the following mismatch repair genes have been demonstrated- MLH 1, MSH 2, PMS 2 or MSH 6.

ENVIRONMENTAL FACTORS:

Age – risk increase proportionately with in age.

Gender – higher the incidence in males[19].

Inflammatory Bowel Disease – Both ulcerative colitis and Crohn's disease can cause colorectal cancer[20]. Extent of the disease, duration and activity are the determinants[21].Ureterocolic anastomosis, long term immunosuppression[22], following organ transplantation, insulin resistant Diabetes Mellitus (due to long-term effects of insulin-like growth factor)[23,24], pelvic irradiation[25] predispose to colorectal carcinoma.

LIFESTYLE RELATED FACTORS:

Consumption of fresh red meat and processed meat is associated with Increase in risk[26,27,28]. Alcohol is a risk factor and reduction in alcohol consumption may reduce the incidence of occurrence of colorectal cancer, especially in those with a positive family history of colorectal carcinoma[29].There is an inverse association with vegetable and fiber intake[30,31]. Obesity[32], cigarette smoking[33], sedentary lifestyle[34], increase the risk. Folate ingestion reduces the risk[35]. Use of NSAIDS, especially daily intake of low-dose aspirin reduces the incidence of colorectal cancer[36.]

OverexpressionCOX-2 is seen in 90% colorectal carcinomas and 40-90% adenomas. COX-2 is needed for prostaglandin E2 synthesis which in turn leads to epithelial proliferation after epithelial injury. N-SAIDS inhibit COX-2, thereby reducing PGE-2 production and so the incidence of colorectal carcinoma[37].

ANATOMY, EMBRYOLOGY & PHYSIOLOGY:

It is necessary to study the difference between the segments of the large bowel for better understanding of the mechanism of colorectal cancer. The large intestine is from the distal end of ileum to anus and measures about 1.5m; It consists of caecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum. The junction between ascending colon and transverse colon is called hepatic flexure and the junction between transverse colon and descending flexure is known as splenic flexure. Rectum measures about 8-15 cm, lies in the pelvis and ends at the anal canal.

General characters of the large intestine are its large internal diameter compared to that of the small intestine and the presence of omental appendices which are peritoneum covered accumulations of fat. The longitudinal muscle layer is segregated into 3 narrow bands called taeniae coli, which are prominent in the caecum and colon, less visible in the rectum. Haustrations represent the sacculations of the colon. Mucosa of the colon appears flat as there are no villi. Numerous non-branching crypts are seen punctuating the mucosa.

The proximal and distal colon are intraperitoneal whereas rectum is retroperitoneal. Proximal colon embryologically develops from the midgut, nourished by superior mesenteric artery innervated by vague nerve. The capillary network of the proximal colon is multilayered with increased capillary width to absorb water and electrolytes[38,39.]

Distal colon develops from the hindgut, nourished by the branches of inferior mesenteric artery, innervated by S2-S4 nerves. The capillary network is single-layered. Wall of the colon is thinner when compared to rectum when visualized by endoscopic ultrasonography with a higher average crypt length[40.]

Histologically, the bowel wall consists of four layers namely mucosa, submucosa, muscularis proproia or muscularis externa and serosa. The mucosal surface is lined by a single layer of columnar cells. The surface epithelium is composed of absorptive cells and mucin secreting goblet cells. The epithelial cells rests on a thin basement membrane. The crypts of Lieberkühn open directly on to the surface. The crypts have a test-tube like shape and are arranged parallel to each other.

Immunohistochemically, the epithelial cells of the normal colonic mucosa contain CK 18,CK 19, CK 20 but not CK 7.Lamina propria consists of collagen fibers, vessels, nerves, smooth muscle bundles, few lymphocytes, plasma cells and histiocytes.

The submucosa contains loose connective tissue with vessels and nerves. Submucosal layer contains Meissner's plexus of nerves. Muscularis propria consists of inner circular and outer longitudinal layers. Auerbach's myenteric plexus of nerves are present in between these two muscular layers.

Serosa is composed of single layer of flattened mesothelial cells. Mucin secreting goblet cells are higher in rectum and sigmoid colon and there is a high concentration of endocrine cells in the rectum. In descending colon, neutral mucin is predominant, whereas in rectum acidic mucins predominate[41].

SITES OF COLORECTAL MALIGNANCIES:

Rectum and sigmoid colon are the most common sites for colorectal carcinomas. In recent years, there is an increase in the incidence of right sided colonic cancer[42,43.]

Females, history of cholecystectomy, hormone therapy for breast cancer, multiparity are some of the factors increasing the risk, possibly due to a change in metabolism of bile acids either in the component or quality.

PATHOGENESIS OF COLORECTAL CARCINOMAS:

Most colorectal carcinomas arise from adenomas[44]. Residual adenoma can be identified in about 10-30% cases while the remainder are overgrown, and the precursor lesion is not apparent histologically[45]. Adenomas precede cancer by 15 years[46].

ABERRANT CRYPT FOCI:

It is the earliest morphological precursor of epithelial neoplasia.Aberrant crypt foci have crypts of enlarged caliber and thickened epithelium with mucin depletion[47]. There are two subtypes:

- Hyperplastic type with RAS protooncogene mutation.
- Dysplastic type with APC gene mutation

They represent the intermediate step between normal colonic epithelium and grossly apparent adenomatous growth[48].

GENETIC MODEL:

Accumulation of genetic alterations leads to progression of adenoma to Carcinoma[49,50,51].

GENOME INSTABILITY:

It refers to increased acquisition and tolerance of mutation by the cells – which is a hallmark of colorectal cancer development[52,53].

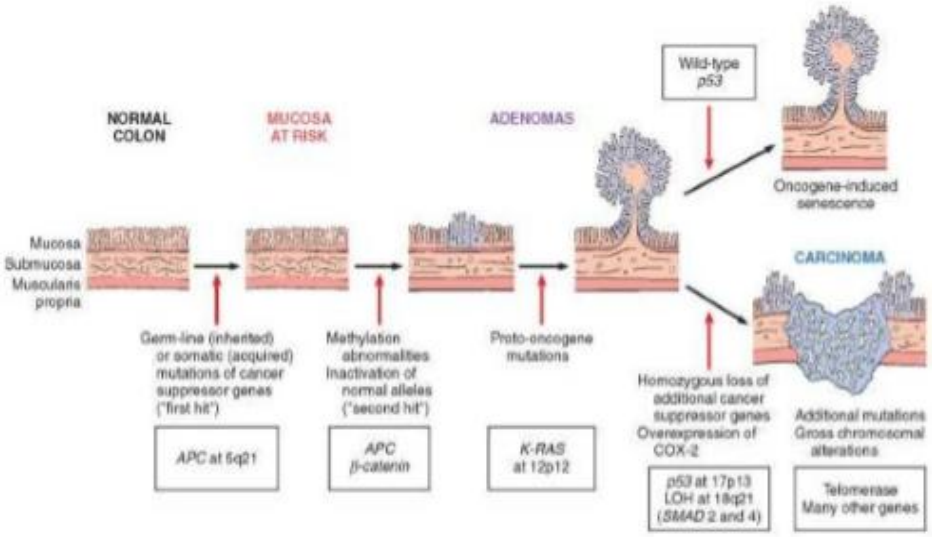
Genomic Instability is subdivided into:

- Chromosome instability
- Microsatellite instability

Accounting for 85% and 15% colon and rectal carcinomas respectively was first identified as the gene mutated in FAP[54].Sporadic colorectal cancers can also harbor APC mutation[55].This APC is known as the gatekeeper gene of colorectal neoplasia. Other genetic alterations are the presence of activating KRAS mutations in about 35% of cases, leading to uncontrolled growth and reduced apoptosis[56]. Due to chromosomal deletions, TP53inactivation occurs is 50-70% cases[57].

ADENOMA-CARCINOMA SEQUENCE:

It accounts for 80% of sporadic colon tumors



Molecular basis for the evolution of colorectal cancer through the adeno-ma- carcinoma sequence

APC-BETA-CATENIN PATHWAY:

APC /β –catenin/TCF pathway deregulation play a central role colorectal cancer.βcatenin gene is regulated by APC protein which is mutated in 80% of sporadic colon cancers. Also, APC gene mutation is seen in FAP associated carcinoma.

Under physiological condition large fraction of β catenin is bound to cell membrane with E cadherin which is cell to cell adhesion protein and through α catenin to actin cytoskeleton. β catenin is incorporated into large protein complex including APC,tumor suppressor protein, glycogen synthase kinase and proteins like axins and conductins. phosphorylation by GSK3β leads to ubiqui-

tination and degradation by ubiquitin – proteasome pathway. Recent studies have shown that there is existence of APC /axins – independent pathway for βcatenin phosphorylation.

Inhibition of the GSK 3β leads to dissociation of APC /axins/ β catenin complex and cytosolic β catenin accumulation[58.]

The products of these gene are involved in cell proliferation and promote cancer development. Recently it has been shown that down regulation of β-catenin expression by accelerating its proteasomal degradation led to impaired cell proliferation and abolished the tumorigenic potential. Celecoxib was described to have antineoplastic effects especially on tumors arising from dysregulation of APC / β-catenin pathway[59].

ADENOMA:

Adenoma is a benign epithelial tumor of colon with potential of malignant transformation.adenomas are common in males, but malignant transformation is more common in females because adenoma is larger and more dysplastic in females.Adenoma is uncommon before 40 years of age.size and multiplicity of adenoma are age related.adenoma occurs more frequently in distal colon and rectum.

Usually adenomas are asymptomatic, but large adenomas can cause occult bleeding and frank bleeding.villous adenoma causes increases mucus secretion which leads to water and electrolyte imbalance.autosomal dominant type of inheritance is seen in familial inheritance of adenoma.

GROSS:

Adenomas may be sessile or polypoidal same color as that of the surrounding normal mucosa.When the size of the nodule increases they become polypoidal with their head broken into multiple nodules which gives them a baby cauliflower like appearance.

Sessile adenomas are less circumscribed compared with the pedunculated adenoma, so the higher chance of recurrence.

FIG: 1 ADENOMA – GROSS

HISTOPATHOLOGY:

Adenoma may be tubular or villous type with the lining epithelium which has stratified, hyperchromatic, pleomorphic nuclei.Mitotic activity is not only seen in the basal layer but also in surface epithelium. The glands are tightly packed coiled and branched. Paneth and endocrine cells are distributed among them.Based on their architecture the adenomas are classified into tubular, villous and tubulovillous. Tubular adenoma is composed of more than 80% branching tubules in the lamina propria.

- Villous adenoma is composed 80% of leaf like or finger like component in the lamina propria.villous adenoma is sessile and larger in diameter.
- Tubulovillous adenoma has both tubular and villous component but both constituting less than 20% of the adenoma.

Based on the epithelial dysplasia they are classified as low grade and high-grade dysplasia. Low grade dysplasia has low nucleo cytoplasmic ratio, nuclear stratification,crowding and elongated nucleus.Mucin secretion is preserved but reduced.In high grade dysplasia the nucleo cytoplasmic ratio is increased,hyperchromatic nuclei with prominent nucleoli is seen.glands are arranged in back to back pattern.

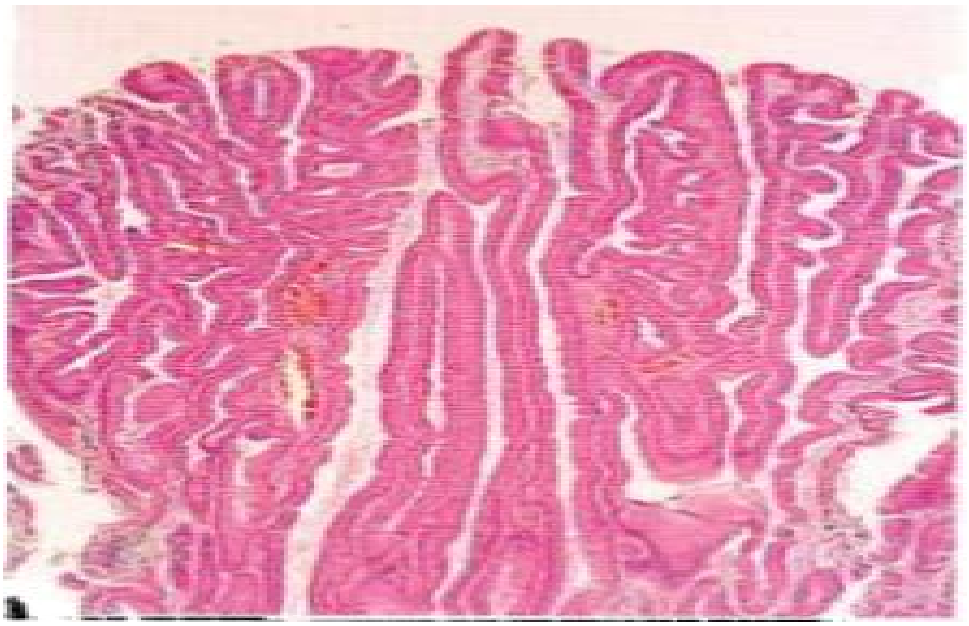


FIG 2: VILLOUS ADENOMA

MICROSATELLITE INSTABILITY:

Mutations accumulate in microsatellite repeats, in patients with DNAmismatch repair deficiency and this is referred to as microsatellite instability. When these mutations involve the coding / promoter region of the gene responsible for reputation of cell growth, uncontrolled cell proliferation occurs, and increased survival of genetically abnormal clones occurs.

BRAF mutations can also occurs. Combination of MSI, BRAF mutation and methylation of specific targets are the hallmarks of this pathway.Large intestinal cancers in a panel of 5 microsatellites (BAT 25, BAT 26, D5S346,D2S123, D17S250)[60].

- When instability occurs in any of the 2 microsatellites, it is called MSI-H
- If instability occurs in only one microsatellite it is called MSI-L.

GENETIC SUSCEPTIBILITY:

Colorectal carcinomas can be associated with polyposis syndromes and non-polyposis syndromes.

ADENOMATOUS POLYPOSIS SYNDROMES:

FAMILIAL ADENOMATOUS POLYPOSIS:

It is an autosomal dominant condition. Mutated APC gene can be inherited, or new germline mutation of APC can occur[61]. Adenomas occur because of loss of second APC allele within colonic epithelial cells[62].FAP is defined as the presence of >100 adenomatous polyps in the colon, many patients have several hundreds to thousands of polyps. Adenocarcinomas occurs in mid-30s and as early as 17 years.FAP accounts for about 1% of colon cancers. Equal sex predilection is seen, and the frequency of occurrence is about 1 in 8000 – 14000 in general population[63]. In FAP, COX2 inhibitors play a role in reducing polyp burden[64].

SUBTYPES:

Gardner syndrome, Turcot syndrome, attenuated FAP. Treatment includes screening during adolescence and post adolescent prophylactic colectomy.

GARDNER'S SYNDROME:

Patients with FAP in addition to those in GI tract, have other manifestations said to have Gardner's syndrome[65,66,67.]Clinical features include epidermal cysts, dental abnormalities,osteomas of mandible, skull and long bones, aggressive desmoid tumors and congenital hypertrophy of retinal pigment epithelium.

TURCOT SYNDROME:

It refers to the coexistence of hereditary colon cancer syndrome (FAP /HNPCC) along with CNS Tumor[68,69], like medulloblastoma, astrocytoma and ependymoma. Distinct germline defects in either APC (in FAP) / DNAmismatch repair genes (in HNPCC).

ATTENUATED FAP:

Polyps are less than 100 in number (usually <30)[70,71]. Adenomas andAdenocarcinomas are seen to develop in later stages in life than in classic FAP. But lifetime risk of developing malignancy is 80%.

HNPCC / LYNCH SYNDROME:

It has autosomal dominant type of inheritance.Inherited defects in at least one of the family of DNA mismatch repair enzymes are present (hMLH, hMSH2, hMSH6, hPMS2)[72,73] which leads to microsatellite instability and rapid accumulation of somatic mutations in genes that control tumor progression. Risk of developing colon cancer is 80-90%.

HNPCC constitutes about 5% of all colonic cancers, with a right sided preponderance at an early age. The tumors have signet-ring / mucinous component with a microglandular or medullary growth pattern with pushing margins. They are usually poorly differentiated with prominent tumor lymphocytic infiltration.

JUVENILE POLYPOSIS SYNDROME:

It is characterized by the presence of multiple juvenile polyps throughout the bowel. Juvenile polyps are also called retention polyps.Microscopically, juvenile polyps show ulcerated surface with granulation tissue formation with underlying cystically dilated glands filled with mucus and separated by an edematous stroma.This condition is associated with development of multiple adenomatous polyps, leading to adenocarcinoma. Inactivating SMAD 4 mutation have been demonstrated.

CRONKHITE – CANADA SYNDROME:

It is a non- hereditary disorder presents with multiple juvenile polyps associated with ectodermal changes. Adenomatous polyps and adenocarcinoma can develop.

PEUTZ- JEGHERS SYNDROME:

It is characterized by the presence of Peutz – Jegher’s polyps. These polyps are defined by the presence of ramifying smooth muscle fibers from the

muscular is mucosa, in between the glands.Germline mutations of LKB 1 gene have been demonstrated. This syndrome can be associated with adenomatous polyps with high grade dysplasia and adenocarcinoma of the large bowel.

COWDEN SYNDROME:

It is an autosomal dominant condition, also called as multiple hamartoma syndrome, the polyps in this syndrome have disorganized proliferation of the muscularis mucosa.

TORRE- MUIR SYNDROME:

It is an autosomal dominant condition. Around 15% of females with this syndrome develop endometrial cancer and colorectal carcinoma is seen 50% of people with this syndrome, most of which are found in the right colon.

NOTE: Any polyposis syndrome involving large intestine can evolve into a malignancy, FAP and Gardner syndrome having the greatest risk.

SIGNS AND SYMPTOMS:

Haematochezia and anemia are the common presenting features Many patients have altered bowel habits, especially constipation associated with abdominal distention, bowel obstruction or perforation.rectosigmoid growth may produce tenesmus and bleeding per rectum and other nonspecific symptoms.

IMAGING:

Computer assisted tomography, MRI and trans rectal ultrasonography are used to assess the tumor invasion depth and possibility of regional and distant metastasis. Scintigraphy and positron emission tomography are also used to assess the distant metastasis.

ENDOSCOPY:

Allows observation of the entire large bowel mucosa in addition with biopsy or for therapeutic removal can be done by snare polypectomy,endoscopic mucosal resection or sub mucosal dissection for superficial carcinomas and adenoma.

GROSS APPEARANCE:

The gross appearance of colorectal carcinoma varies depending on the stage of the disease at diagnosis. ‘Small lesions’ may be sessile or pedunculated.

‘Large’ carcinomas can be classified into 4 subtypes.

- Exophytic or polypoid tumors – which can cause obstruction and occur commonly in the caecum.
- Annular or constricting tumors – proximal fragment is dilated with flattened mucosa. This produces a ‘apple core appearance’ on radiography and commonly presents as obstruction.

- Infiltrative and ulcerating tumors – they are often raised, with irregular edges and central excavated area infiltrating into deep layers of bowel wall.
- Diffuse tumors – this subtype is similar to linitis plastica of stomach with diffuse flattening and thickening of colon.

Cut section of the tumor appears homogenous admixed with areas of necrosis. Dilatation can occur because of obstruction and retraction of serosa occurs due to invasion of tumor into muscular is propria or subserosa.

FIG 3: ADENOCARCINOMA – GROSS

MICROSCOPIC APPEARANCE:

CRITERIA FOR MALIGNANCY:

Intramucosal adenocarcinoma refers to the malignancy limited to the lamina propria and muscularis mucosa. It is never associated with lymph node metastasis [74] due to the relative paucity of lymphatics. Intramucosal carcinomas are denoted as Tis. Therefore, invasion into submucosa is requiredto call the colorectal carcinomas as T1.

DIAGNOSIS BY BIOPSY:

Endoscopic biopsies are used for diagnosis. To determine the presence of invasion is the most important aspect of pathological examination. WHOclassification of tumors of colon and rectum are given in annexure II.Staging of colorectal carcinoma given in the annexure III

ADENOCARCINOMA:

Colonic adenocarcinoma is usually moderately differentiated, the neoplastic cells being arranged as medium to large sized glandular pattern with variability in their size and configuration with a moderate amount of stroma.Well differentiated tumors contain tall and columnar epithelial cells were as polygonal or cuboidal with decreasing degrees of differentiation.Numerous mitotic figures are present.

DIRTY NECROSIS:

Refers to the presence of inspissated eosinophilic mucus, nuclear andcellular debris within the glandular lumen. Thus, when dirty necrosis is present in a metastasis from an unknown primary, it is worth to search for a colorectal primary.

Leading edge of the tumor is usually having the infiltrating glands.Desmoplastic reaction in the stroma may be prominent. Presence of other cells in variable amounts, for example, Paneth cells, neuroendocrinecells, squamous cells, trophoblasts are of no prognostic significance.

Grading:

Grading is primarily based on the population of the neoplastic cells that is forms glands when compared to the areas with solid nests or cords of cells with Lumina. Grading system endorsed by AJCC and WHO are usedcommonly[74,75]. The tumors are graded depending up on the amount of differentiation. When>95% of the tumor cells are arranged as glands, they are said to be well differentiated. When<5% tumor cells are arranged as glands, they are called poorly differentiated tumors. When there is no apparent gland formation; they are known as undifferentiated tumors.

According to this grading system, well, moderate and poorly differentiated tumors amount to 10%, 70%, and 20% cases respectively. The diagnosis of poorly differentiated adenocarcinoma has the highest rate of reproducibility and it is the one with a poor survival rate.

FIG 4: Well differentiated adenocarcinoma

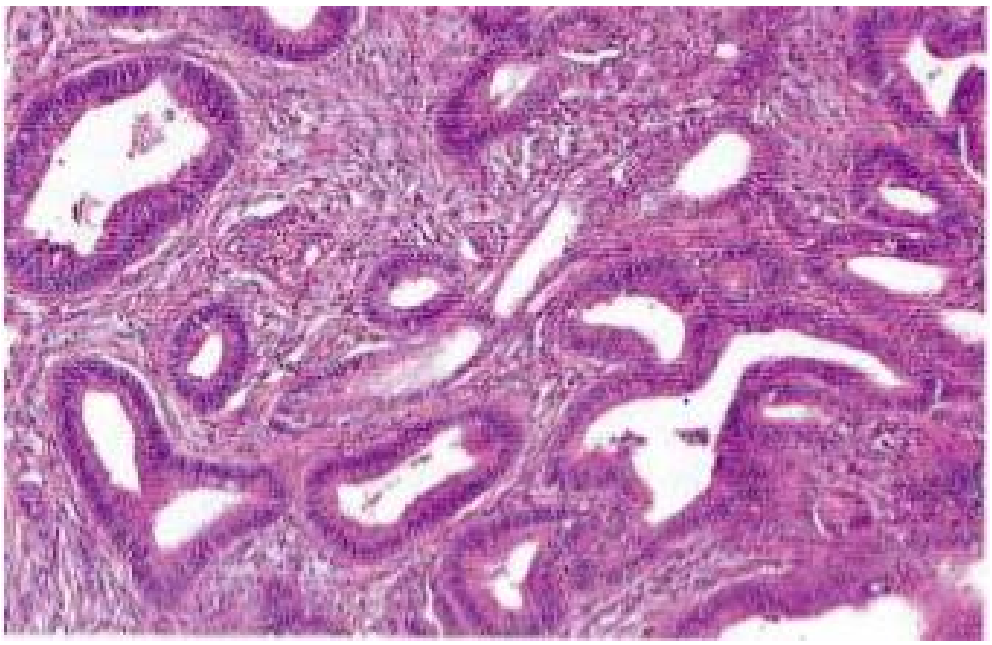
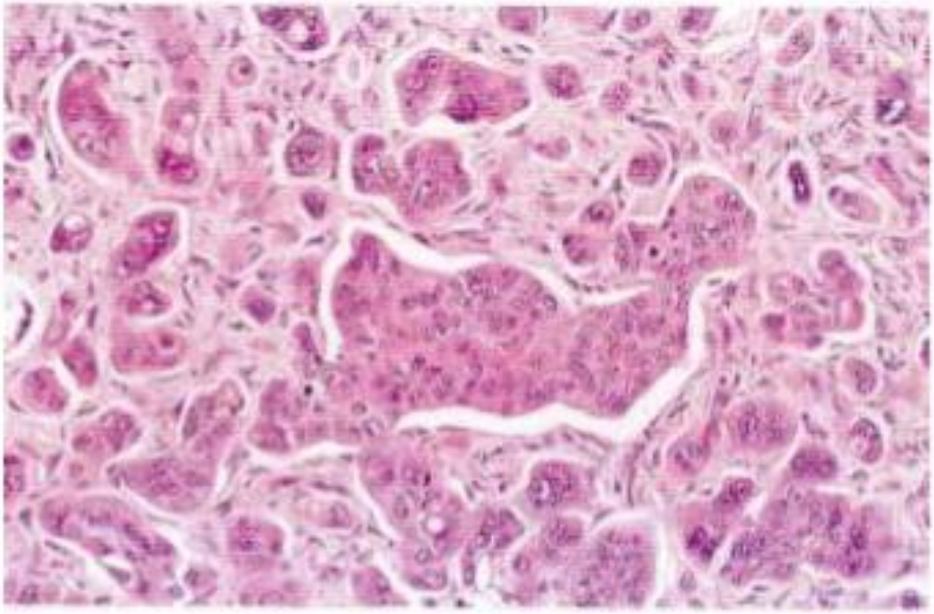


FIG 5: Poorly differentiated adenocarcinoma

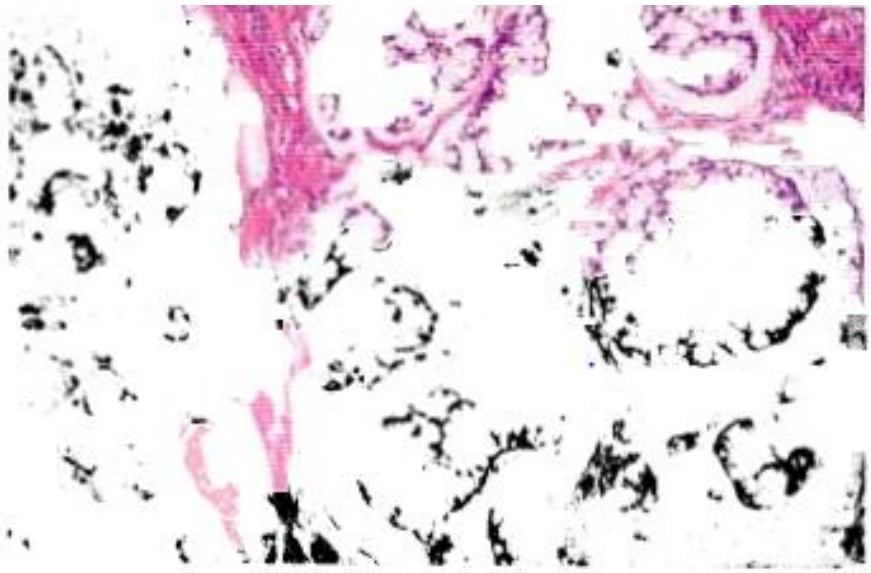


Mucinous adenocarcinoma:

The term mucinous carcinoma means the tumors composed of >50%extracellular mucin. When the mucinous component is >10% or <50%, the tumors are referred to as adenocarcinoma with mucinous differentiation. Mucinous carcinomas contain strips of epithelial cells floating in extracellular pools of mucin and a variable numbers of signet ring cells may also be present. They constitute about 10% of all colonic cancers. They are common in patients with HNPCC and tend to present at a later stage. Microsatellite instability and defects in DNA mismatch repair are common[76].Grossly, they are exophytic. Cut surface is soft and gelatinous. Paucity of fibrous tissue imparts a 'col-loid'appearance to the cut surface. Expression of HATH1, a transcription factor in activation of MUC2 in colonic epithelium is a possible biologic basis for mucinous tumors. Mucinous carcinomas mostly present as right sided colonic tumor.

Microscopically, mucinous adenocarcinomas have abundant large glan-dular structures embedded in extracellular pools of mucin. The mucin shows positivity with Alcian blue or PAS stains. The tumor with infiltrative margin has the poor Prognosis[77,78,79].They may also develop peritoneal implants[80]and have a property to invade adjacent viscera[81] and involve lymph nodes beyond the peri colonic region.

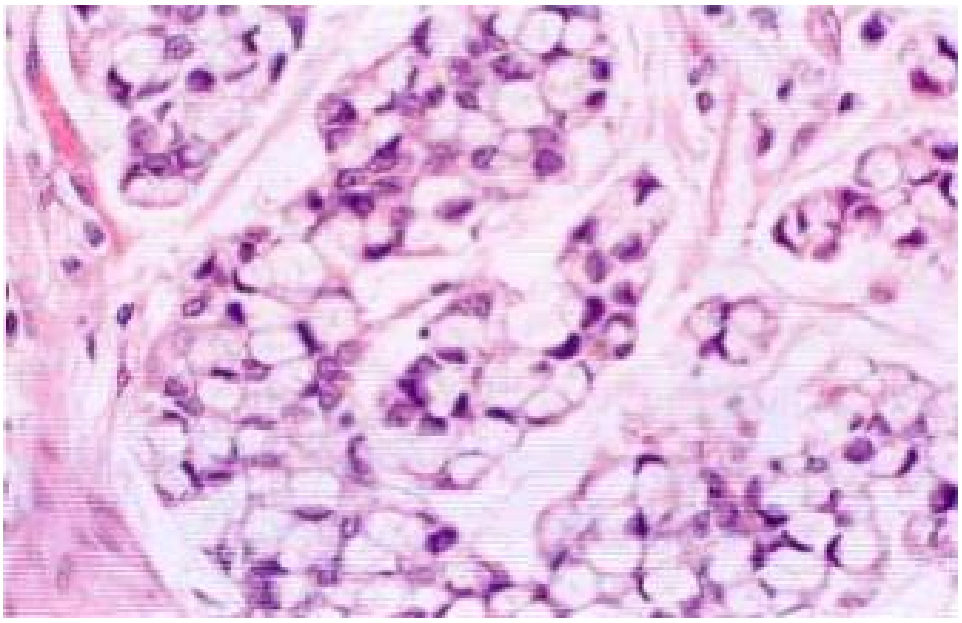
FIG 6: Mucinous carcinoma



Signet ring cell adenocarcinoma

They constitute about 0.5-1% of colorectal carcinomas and contains at least 50% signet ring cells. They are more common among males and mean age of occurrence is 64 years[82].IBD is associated with this tumor[83]. They occur in equal frequency in right and left colon. Grossly, they present as a ulcerative mass.Microscopically, the tumor cells have mucin vacuole, which pushes the nuclei to the periphery of the cytoplasm. The mucin in MUC-2 positive[84]. The patients present at a later stage[85]. Peritoneal seeding is common and 5 years survival is less than 10%.

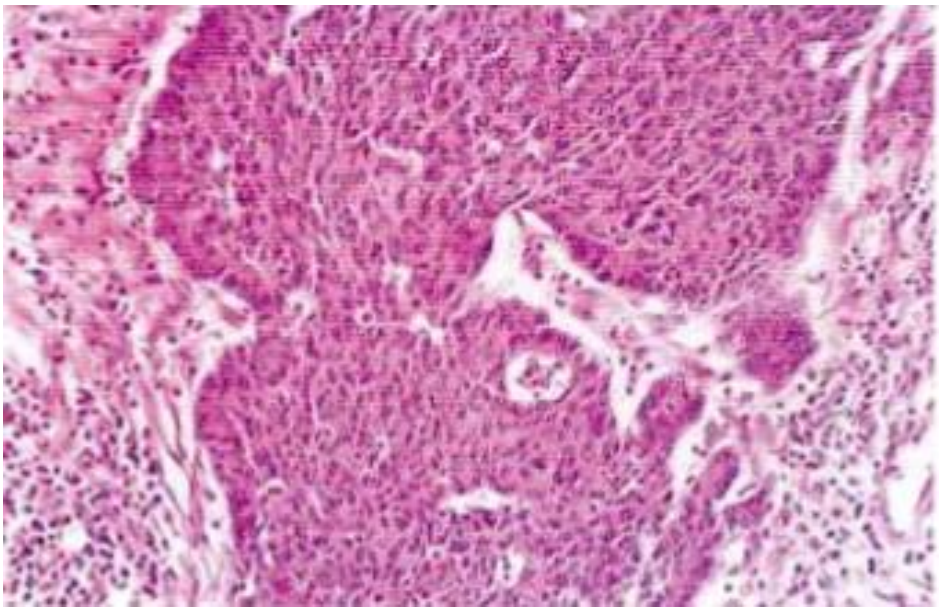
FIG 7: Signet ring cell adenocarcinoma



Undifferentiated carcinomas

These tumors show epithelial differentiation but there is no obvious gland formation or there is less than 5% gland formation. Grossly, they are bulky due to increased cellularity and soft due to lack of desmoplastic, contain extensive areas of necrosis. Microscopically, they have an infiltrative type of growth pattern. The tumor cells are arranged as sheets, cords and trabeculae with variable degrees of anaplasia.

FIG 8: Undifferentiated carcinoma



Medullary carcinoma

Also known as large cell minimally differentiated carcinomas[86]. It was first described by Gessures and co-workers[87].The neoplastic cells have abundant cytoplasm, vesicular nuclei and prominent nucleoli and is associated with marked tumor infiltrating lymphocyte response. These tumors show female preponderance, common in caecum or proximal colon and associated with DNA mismatch repair. They are negative for CK20 and positive for CK7. They have a favorable outcome

Adeno squamous carcinoma

It constitutes about 0.06% of all colorectal cancer. This variant is associated with paraneoplastic hypercalcemia and PTH rP[88,89]. IBD is increases the risk[90,91] Occurrence is right and left colon. Overall 5 years survival rate is 3.1%[92].

Squamous Cell Carcinoma

It accounts of 0.1% of all colorectal cancer cases. Probable histogenesis is from a pluripotent stem cell[93,94] capable of multidirectional differentiation.HPV plays a role in pathogenesis of the tumor[95].

Criteria for diagnosing primary SCC in colon

- To exclude metastasis from other sites.
- Extension from carcinoma of anus.
- An associated squamous – lined fistulous tract must be excluded.

Micropapillary carcinoma

It is a rare variant with a aggressive behavior. Microscopically, the neoplastic cells are arranged as balls or clusters and the cells have eosinophilic cytoplasm and vesicular nuclei. Cleft like spaces are seen around the tumor cells. The micropapillary component ranges from 5-80%. This variant is associated with high incidence of nodal and distant metastasis.

The micropapillary pattern can be mistaken for tumor budding. But the tumor cell nests of micropapillary carcinoma are larger than that of budding[96,97].

Small Cell Variant

Constitutes <1% of all colonic cancers[98]. Microscopically they are same as that of small cell carcinoma of lung. One third of these cases arise from typical adenomas. Areas of squamous differentiation are also present. Expresses NSE, synaptophysin, chromagranin and has an extremely poor prognosis.

Serrated Adenocarcinoma

It refers to cancers arising from sessile serrated polyps or serratedAdenomas[99]. About 7.5% of all colon cancers are associated with serrated precursor lesions[100]. Characteristic features of serrated carcinomas include serration of the glandular lining epithelium, cells with abundant eosinophilic cytoplasm and nuclei with peripheral condensation of chromatin.

Subtypes included:

- Proximal MSI-H cancers arising from sessile serrated polyps.
- Distal MSI-H cancers arising from serrated adenomas.

Other Variants:

Carcinomas with spindle cell component are called sarcomatoid carcinoma. The spindle cells are immunoreactive for keratin.Carcinosarcoma refers to malignant tumors with both carcinomatous and heterologous mesenchymal elements.Rare histopathological variants of colorectal carcinoma includes pigmented, clear cell, pleomorphic, paneth cell rich variants.

Colorectal cancer in patients younger than 40 years

It accounts for 1-2% of all colorectal cancers. 21% of cases have some predisposing factor [101]. The patients present at an advanced stage,most are <40 years and have regional or distant metastasis at the time of diagnosis[102,103]. Common histological variants include the mucinous adenocarcinoma and signet ring cell type tumors. Prognosis is poor in these cases.

IMMUNOPHENOTYPE

It is used to differentiate between primary and secondary tumors and for the characterization of several subtypes.CEA is the most commonly used stain to identify primary colonic cancer.CK-7 and CK-20 can also be used Villin is a protein that shows intestinal differentiation[104].CDX2, transcriptional factor is expressed in normal crypt epithelium and in 90% of colorectal adenocarcinomas[105,106] . There is a strong relationship between tumor stage and CDX2 expression.MUC-2 is also a specific marker for colorectal carcinoma.

COLORECTAL CANCER SCREENING:

It is done using many tests like stool examination for occult blood, colonoscopy, flexible sigmoidoscopy, radiological tests comprising of double contrast barium enema and CT colonography. Screening for colorectal cancer is recommended for men and women more than 40 years of age as part of their routine annual check-up.

PROGNOSIS:

5 years survival rate for colorectal carcinoma, after curative resection is 40-60%[107,108,109]. Two thirds of regional lymph node metastasis or local recurrences are evident with in first 2 years[110].

PROGNOSTIC FACTORS:

Age:

Extremes of age are associated with poor prognosis[111].

Gender:

Females have better prognosis.

Tumor location:

Tumors of left colon have a favorable outcome while those arising in sigmoid colon and rectum have a worse outcome[112].

Serum CEA levels:

Serum CEA levels >5ug/ml is associated with bad prognosis[113,114].

Local Extent:

Tumors extending beyond the bowel wall have worse prognosis.

Tumor Size:

Size of the tumor is related with prognosis[115]but not with metastasis to regional lymph nodes[116].

Tumor edge:

Tumors with non-polypoidal edge has good prognosis[117,118].

Obstruction:

Tumors which presents as an obstruction have a poor prognosis [119,120].

Perforation:

Extensive bowel wall infiltration by the tumor leads to perforation,which is associated with a poor prognosis[121].

Tumor Margins:

Tumors with pushing margins have a good prognosis[122,123].

Tumor budding:

It is defined as the presence of individual tumor cells or tumor cell clusters of more than five cells at the invasive edge of the tumor, which migrate into the desmoplastic stroma[124]. It associated with a poor outcome[125,126].

Vascular invasion:

It shows significant increase in incidence of distant metastasis.Extramural venous invasion is an independent prognostic factor[127,128].

Angiogenesis:

Increased tumor angiogenesis is associated with recurrence and reduced survival. Perineural invasion when present is associated with bad prognosis.

Lymph node involvement

Adequacy of pathological examination is evaluated by the total number of lymph nodes sampled[129]. Lymph node metastasis away from the primary tumor usually have the poor outcome.

Micro metastasis means solitary lymph node metastasis <2mm in size[130,131].

SPECIAL TECHNIQUES TO IMPROVE LYMPH NODE DISSECION

Sentinel lymph node examination:

They are the nodes which are the most direct drainage from the tumor.Therefore, examination of the sentinel lymph nodes helps in identifying patients who have primary tumor limited to the bowel wall and having unnoticed metastasis at the time of surgical resection.Sentinel lymph nodes are identified by injecting a blue dye in the sub serosal layer. One to four lymph nodes that change color first is the sentinel node.

Host lymphatic response:

Host immunological and inflammatory reactions occur in response to colorectal cancer, which includes peritumoral lymphocytes, tumor infiltrating lymphocytes, reactive hyperplasia of regional lymph nodes etc. It acts as an independent prognostic factor[132,133].Tumor infiltrating lymphocytes are seen in colorectal cancers containing DNA mismatch repair deficiency[134].5-7 IELS/HPF is the cut off and it is highly specific and sensitive marker for identification of MSI-H cancers

Microscopic tumor types:

Mucinous carcinoma, signet ring cell carcinoma are variants with poor prognosis.

Staging:

Pathological staging is the most important factor to determine the tumor behavior and patient outcome[135].TNM staging are given in annexure III.Pericolonic tumor deposits, indicate a poor prognostic factor[136].

Margin Status:

The presence of tumor in the radial margin is the most significant factor in predicting local reccurence[137,138].

Tumor thickness:

The thickness of tumor in the central depressed corresponds to the presence of lymph node and liver metastasis[139].

Liver metastasis:

About 15-25% cases have liver metastasis at the time of diagnosis and 20% patients develop metastasis after treatment of primary tumor[140]. Without treatment, median survival years after detection of liver metastasis is about 9 months[141].In some patients, 5-year survival rates have improved with resection of liver metastasis[142].After resection, the status of resected margins remains the most important prognostic factor[143]. Clearance of >1cm has a better outcome[144].

IMMUNOHISTOCHEMISTRY:

It uses antigen-antibody reaction for localizing specific antigens in tissues or cells.

In 1940, Coons detected antigens in frozen tissue sctions[145]. Taylor and Burns demonstrated antigens in FFPE tissues[146]. The antibody is labeled with an enzyme. The labeled antibody visualized by light microscopy is enabled by using a suitable chromogen substrate. Higher sensitivity is obtained by using detection systems like Avidin-biotin complex, Peroxidase antiperoxidase Biotin-Streptavidin method and polymer based labelling systems.

FLEX Monoclonal Mouse Anti – Human beta catenin,clone betacatenin -1, Ready to use is used in this study.The catenin's are structurally related cytoplasmic proteins which have been classified as alpha,beta,gamma according to their electrophoretic mobility[147,148].The catenin gene is located on chromosome 3p21 and encodes a 88 KDa protein. This cytoplasmic protein is multifunctional, playing an essential role in cadherin mediated anchoring and organization of cytoskeleton.Beta catenin is also involved in regulation of gene expression as a mediator of wnt signaling pathway. Cellular beta catenin levels are tightly regulated by a multi protein complex comprised of serine /threonine kinase GSK3β, the APC tumor suppressor gene product and axin which facilitates phosphorylation and subsequent degradation of beta catenin protein. Dysregulation of beta catenin degradation leads to cytoplasmic accumulation of the protein, followed by translocation to the nucleus.Nuclear beta catenin forms complex with DNA binding proteins such as TCF and LEF,activating gene transcription[149].Reagent is ready to use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/l sodium azide.Immunogen is Recombinant C- Terminal β- catenin –GST fusion protein.In western blotting of human epithelial A431 cells the antibody labels a band corresponding to human beta catenin protein .No cross –reactivity with alpha and gamma catenin was observed[150].It is stored at 2 -8 °c.

The technique of immunohistochemistry are as follows:

- Preparation of adhesive coated slides
- Cutting of 4-5-micron sections
- sections are deparaffinized
- Blocking the endogenous enzymes like peroxidase, alkaline phosphatase to avoid non-specific staining
- Antigen retrieval to unmask the antigen
- various methods can be used for antigen retrieval like use of the water bath, autoclaving, microwave heating or pressure cooker treatment.
- The next step includes blocking of non-specific binding sites
- Binding of primary antibody
- Binding of secondary antibody
- Use of detection methods like peroxidase – antiperoxidase, avidin – biotin conjugates, avidin – streptavidin complexes, polymer based detection systems.
- Addition of chromogen substrate, usually Diaminobenzidine(DAB)
- Counterstaining, dehydrating and mounting the sections.

Quality control in IHC:

Quality control measures should be taken care of during the preanalytical, analytical and post analytical studies.

Use of controls:

Positive control is used to test the presence of antigen, integrity if the antibody and validates the methodology. The test is done on a tissue known to be immunoreactive for a particular primary antibody. It should have positive staining.For the purpose of negative control, the same section used for positive control should be used. The primary antibody is replaced by a non-immune antiserum in the same dilution of the primary antibody.

Recent advances in IHC:

GENOGENIC IMMUNOHISTOCHEMISTRY:

It can be used to trace the molecular changes by IHC. It is used for both diagnosis and therapy. With regard to colon cancer, microsatellite instability can be detected by genogenic immunohistochemistry.

MONOCLONAL ANTIBODY DEVELOPMENT:

Development of highly specific antibodies using recombinant technology has paved way for the make of molecules with high stability, high potency and ultra-high affinity.

AUTOMATION IN IMMUNOHISTOCHEMISTRY:

Automated techniques are available for the IHC procedure and for microscopic image analysis. Computerized image capture and analysis systems are available.at present.

MATERIALS AND METHODS

This study is done at Government Kilpauk Medical College. This is a prospective study done for the period of 3 years from 2016 to 2018Study population is the patients diagnosed as having colorectal adenoma and carcinoma by histopathological examination.

Inclusion Criteria:

Histopathologically proven cases of colorectal adenoma and carcinoma

Exclusion Criteria:

Patients diagnosed other than adenocarcinoma like,

- lymphomas
- Neuroendocrine tumours
- Mesenchymal neoplasms
- Poorly differentiated tumours
- Metastatic tumours

Sample size is 50 colectomy cases.

Data collection and Methodology:

Colorectal carcinoma and adenoma cases reported in the department of pathology are included in this study.Relevant clinical details and clinicopathological variables – Age, Sex, clinical features, Duration, colonoscopic findings, Gross appearance , histopathological diagnosis and grade at the Department of Pathology, Kilpauk Medical College are obtained.

Formalin fixed,paraffin embedded tissue blocks ,and H AND E sections are used to grade colorectal carcinoma and unstained sections are used for immunohistochemically study for the expression of beta catenin and graded appropriately.

Immunohistochemistry Procedure:

Slide Preparation:

Sections with a thickness of 4-5u were cut from FFPE tissue and transferred to slides coated with gelatin and chrome alum.The slides were incubated overnight at 58ºC. The sections were deparaffinised in xylene for 30 minutes (15 minute x 2 changes) The sections were dehydrated with absolute alcohol for 10 minutes (5 minutes x 2 changes). The sections were then rinsed in distilled water for 5 minutes.

Antigen Retrieval

Retrieval buffer was prepared and was preheated for 4 minutes at 800W. The sections were then immersed in the retrieval buffer and incubated in the pressure cooker at medium temperature. The slides were cooled at room temperature, washed with distilled water and then with TBS for 3 minutes (2 changes). Peroxidase block was applied over the sections for 10 minutes and then washed with TBS for 2 minutes (2 changes).

Antibody Application:

The sections were treated with primary antibody for 30 minutes and washed with TBS for 2 minutes (2 changes).

Antibody Application:

The sections were treated with primary antibody for 30 minutes and washed with TBS for 2 minutes (2 changes). They were treated with polyexcel target binder for 15 minutes and washed with TBS for 2 minutes (2 changes). The sections were treated with HRP for 15 minutes and washed with TBS for 2 minutes (2 changes).

Chromogen Application:

DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1ml DAB buffer. DAB substrate solution was applied on the sections for 5 minutes. The slides were washed with distilled water for 2 minutes. The sections were counterstained with hematoxylin for 10 seconds. The slides were washed with distilled water for 5 minutes, air dried, cleared with xylene and mounted with DPX.

Interpretation and scoring system:

The slides were analyzed for the presence of immune histochemical reaction for beta catenin and scoring was done.

0 – negative

1+ circumferential membrane pattern was incomplete

2+ circumferential staining with weak and intermediate intensity

3+ complete strong circumferential staining

Scoring was done separately for membrane,cytoplasm and nucleus.

0 and 1+ was taken as negative.

2+ and 3+ was taken as positive.

Statistical Analysis:

The statistical analysis was done using Statistical Package for Social Science software. The expression of beta catenin was correlated with variables like age, gender, location, size, grade, stage, histological grade, lymph node involvement and lymph vascular invasion

OBSERVATION AND RESULTS

Age Distribution:

In this study age group distribution of colorectal adenoma and carcinoma were from 28 to 72 years. The patients were divided into 6 groups ie (less ≤30 years ,31 -40 years ,41- 50 years,51 – 60years,61 -70 years, ≥ 70 years).

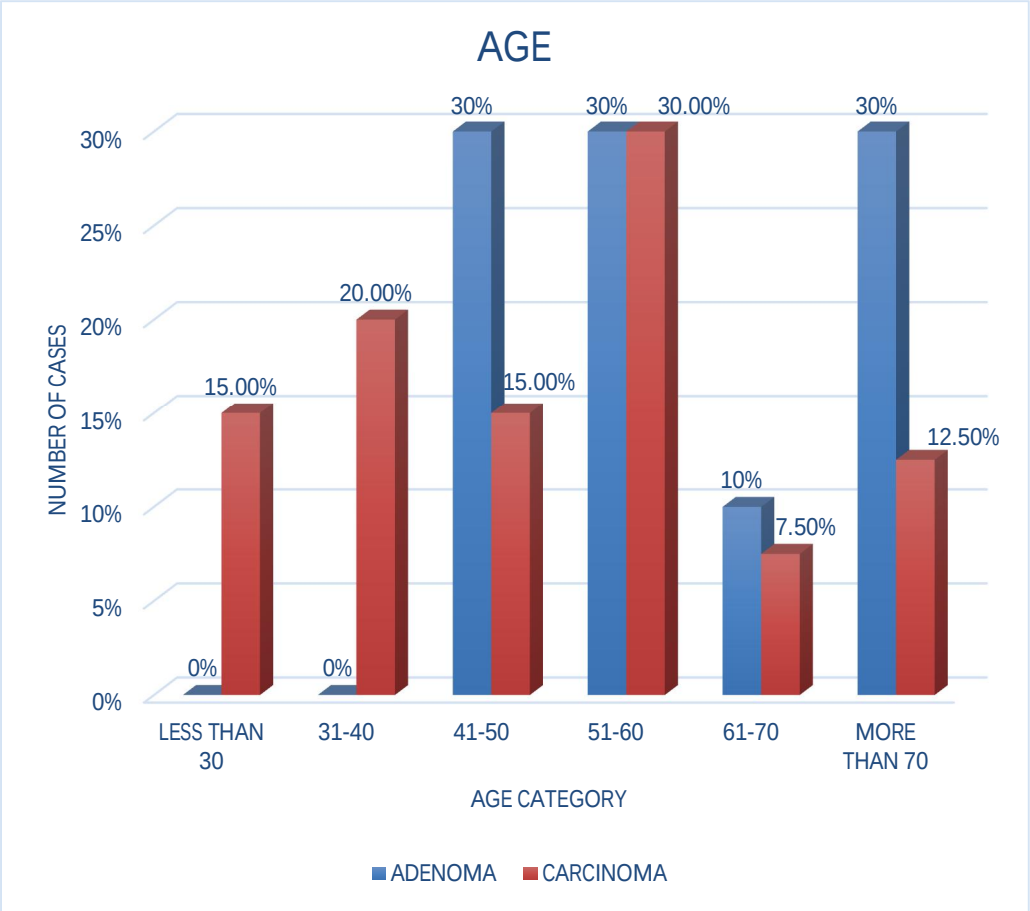
Table 1: Age distribution of colorectal adenoma and carcinoma is shown in the following table.

	Age Distribution			
	Adenoma		**Carcinoma**	
AGE	**no of cases**	**%**	**no of cases**	**%**
<30	0	0%	6	15%
31-40 years	0	0%	8	20%
41-50 years	3	30%	6	15%
51-60 years	3	30%	12	30%
61-70 years	1	10%	3	7.50%
>70 years	3	30%	5	13%
Total	10	100%	40	100%

Pearson chi – square =6.094,P value -0.297

From this table highest incidence of colorectal carcinoma is seen in the age group between 51 – 60years. Colorectal adenomas have no age group of distribution.

Chart 1: Age distribution among the cases



SEX

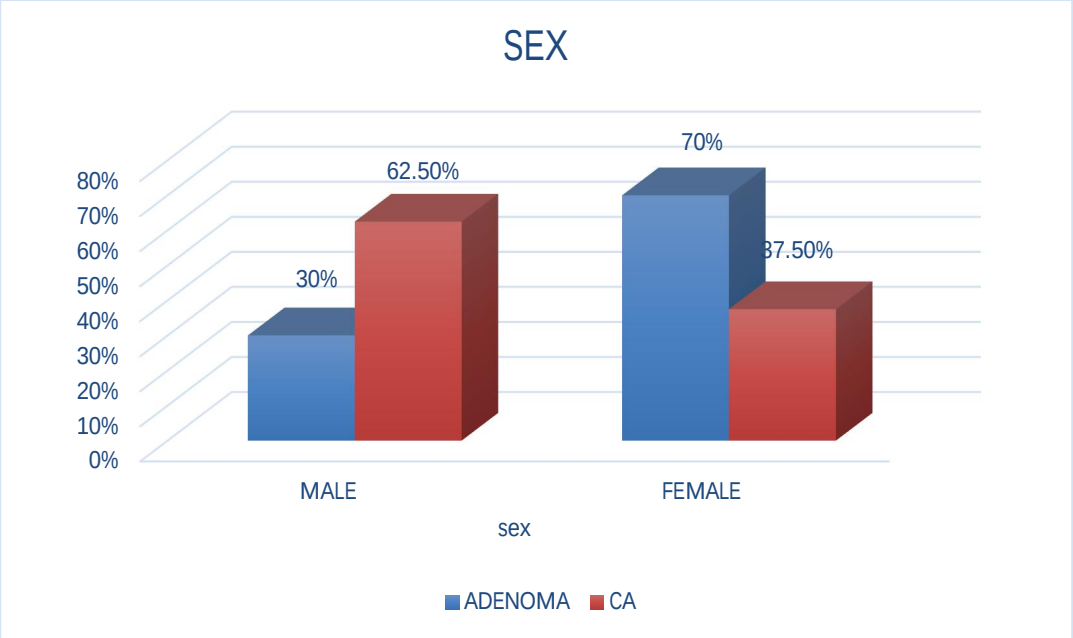
Table 2:Gender wise distribution of colorectal colorectal adenoma and carcinoma

In this study the incidence of colorectal adenoma showed female pre-ponderance, colorectal carcinoma showed male preponderance.

Sex	Type of lesion			
	Adenoma		Carcinoma	
	no of cases	%	no of cases	%
male	3	30%	25	62.5%
female	7	70%	15	37.5%
Total	10	100%	40	100%

Pearson chi square = 3.429 , P value -0.064

Chart 2:Sex wise distribution of colorectal adenoma and carcinoma



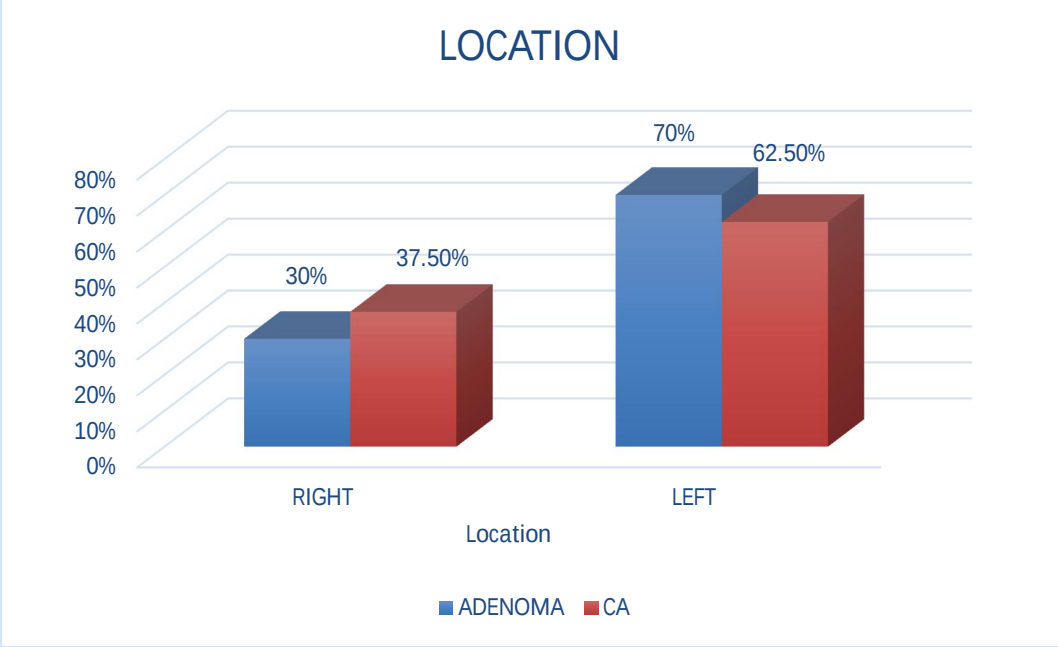
Site wise distribution of adenoma and carcinoma

In this study both the colorectal adenoma and carcinoma showed left sided preponderance.

LOCATION	type of lesion			
	Adenoma		Carcinoma	
	no of cases	%	no of cases	%
RIGHT	3	30%	15	38%
LEFT	7	70%	25	63%
TOTAL	10	100%	40	100%

Pearson chi square = 0.195,P value -0.659

Chart 3 site wise distribution of adenoma and carcinoma



Distribution of colorectal adenoma and carcinoma based on gross appearance

Out of 10 cases of adenoma 6 cases presented as ulcer proliferative growth ,1 as circumferential growth and 3 as polypoidal growth.

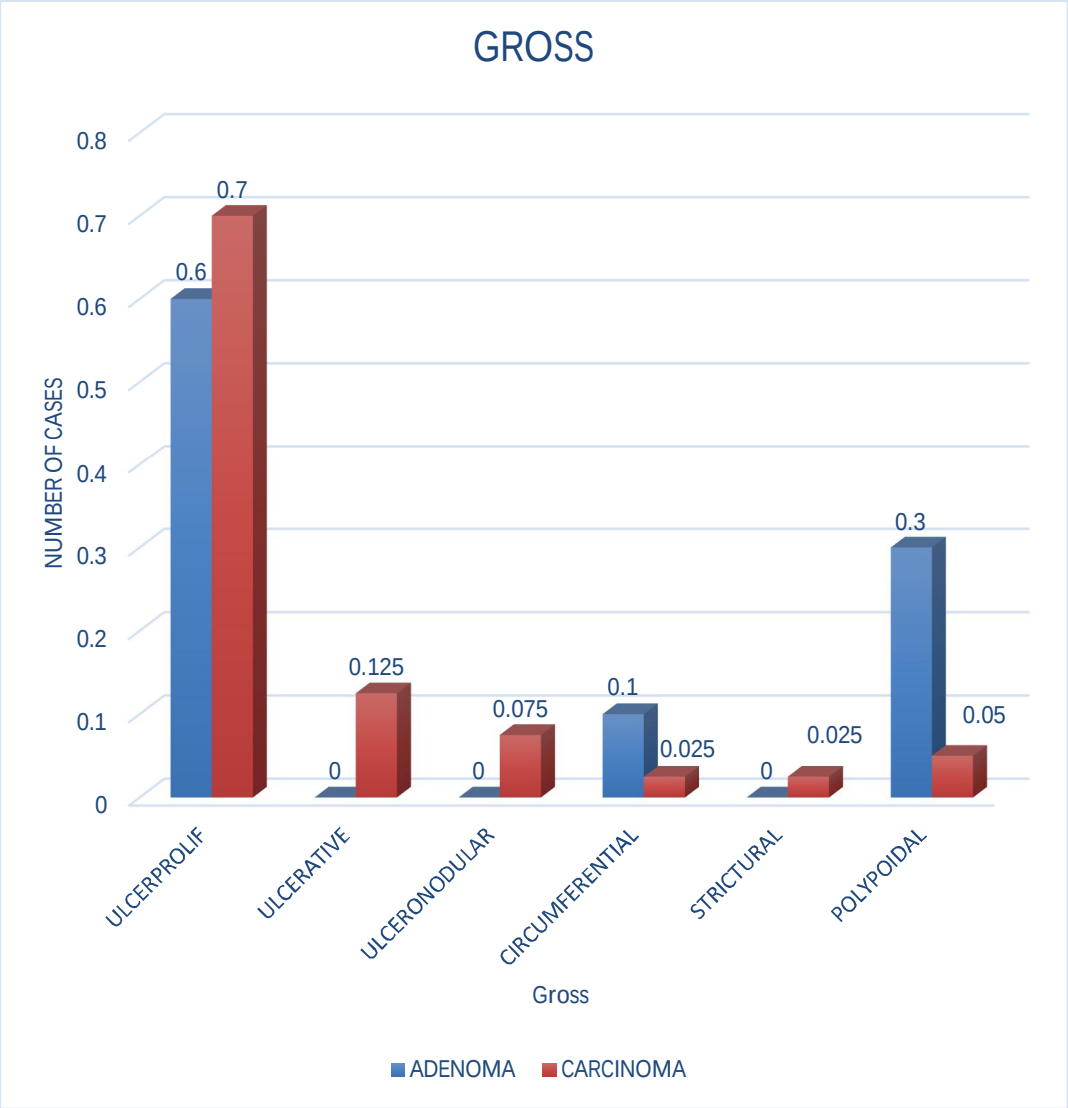
Out of 40 cases of carcinoma 28 cases presented as ulceroprolierative growth ,5 as ulcerative growth,3 as ulcer nodular growth,1 as circumferential growth ,1 as stricture and 2 as polypoidal mass.

Table 4:

Gross	**Gross**			
	Adenoma		**Carcinoma**	
	no of cases	**%**	**no of cases**	**%**
ULCERPROLIF	6	60%	28	70%
ULCERATIVE	0	0%	5	13%
ULCERONODULAR	0	0%	3	8%
CIRCUMFERENTIAL	10	10%	1	3%
STRICTURAL	0	0%	1	3%
POLYPOIDAL	30	30%	2	5%
TOTAL	100	100%	40	100%

Pearson chi square – 8.493,P value – 0.131

Chart 4: Distribution of cases based on gross appearance



Distribution of cases based on histopathological subtypes.

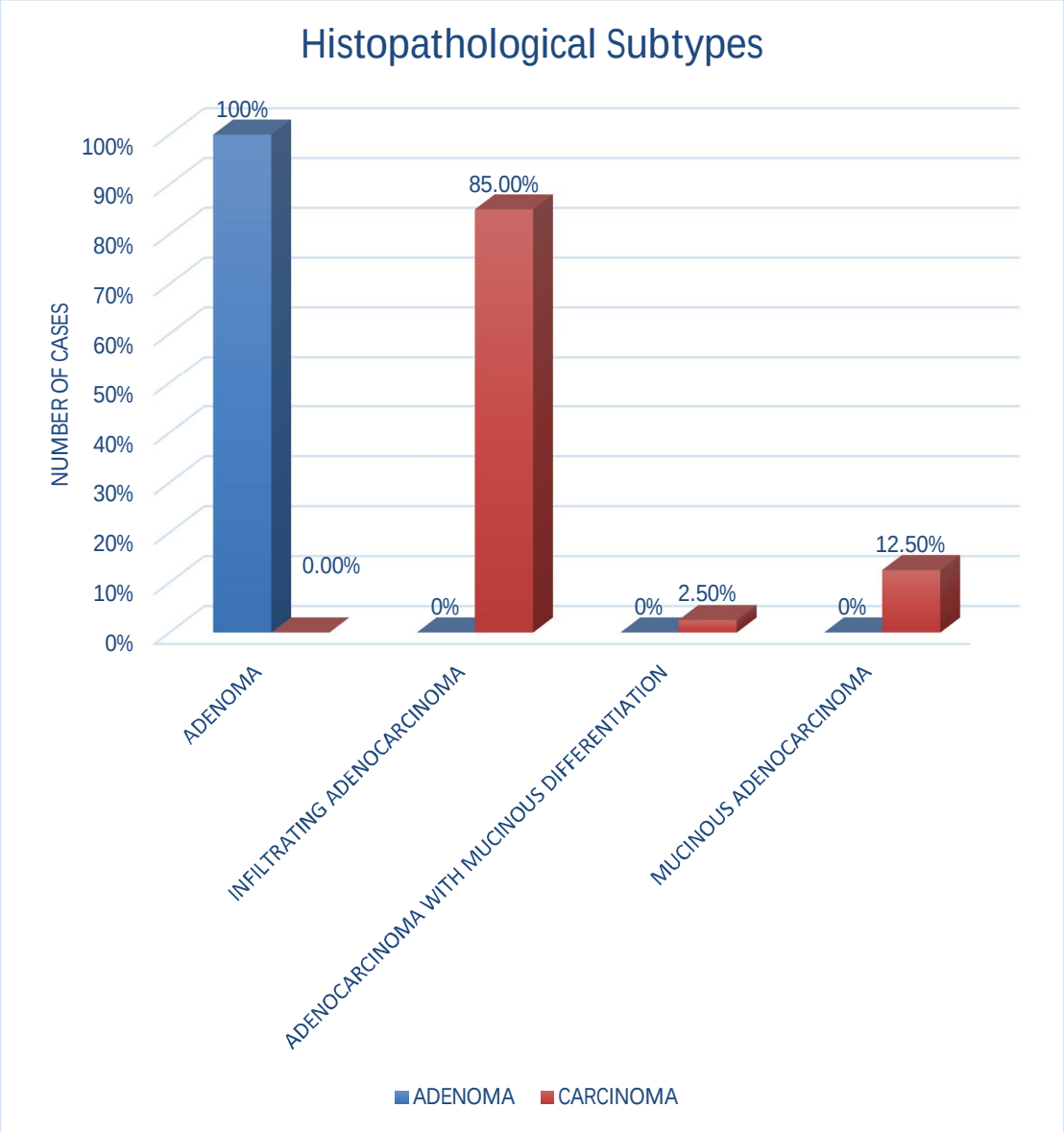
Among 50 cases 10 cases were adenoma ,34 where are infiltrating adeno-carcinoma ,1 case with infiltrating adenocarcinoma with mucinous differentia-tion and 5 cases were mucinous carcinoma.

Table 5:

HPE	**type of lesion**			
	Adenoma		**Carcinoma**	
	no of cases	**%**	**no of cases**	**%**
ADENOMA	10	100%	0	0%
INFILT ADENO-CARCINOMA	0	0%	34	85%
INFLIT CA WITH MUCINOUS	0	0%	1	3%
MUCINOUS ADE-NOCARCINOMA	0	0%	5	13%
TOTAL	10	100%	40	100 %

Pearson chi square = 50.000 ,p value – < 0.01

Chart 5: Distribution of cases based on their histology



Distribution of cases based on grades

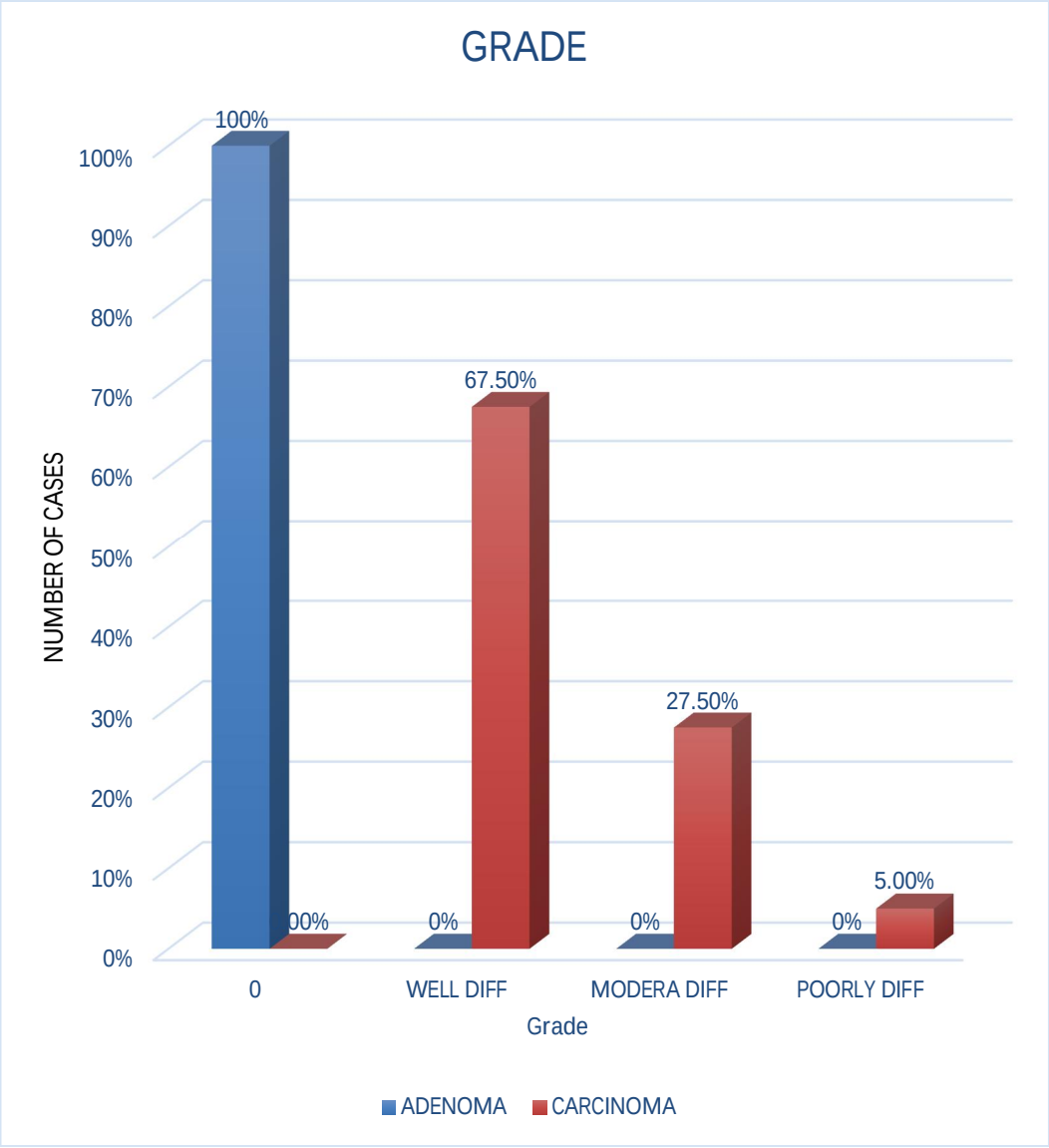
In this study 10 cases of adenoma were graded as grade 0 out of 40 cases of carcinoma 27 cases were well differentiated ,11 cases were moderately differentiated,2 cases were poorly differentiated.

Table 6:

GRADE	type of lesion			
	Adenoma		Carcinoma	
	no of cases	%	no of cases	%
0	10	100%	0	0%
WELL DIFF	0	0%	27	67.5%
MODERA DIFF	0	0%	11	27.5%
POORLY DIFF	0	0%	2	5%
TOTAL	10	100%	40	100%

Pearson chi square =50.000 ,p value – < 0.01

Chart 6:Distribution of cases based on the grade of the lesion



Stage wise distribution of cases

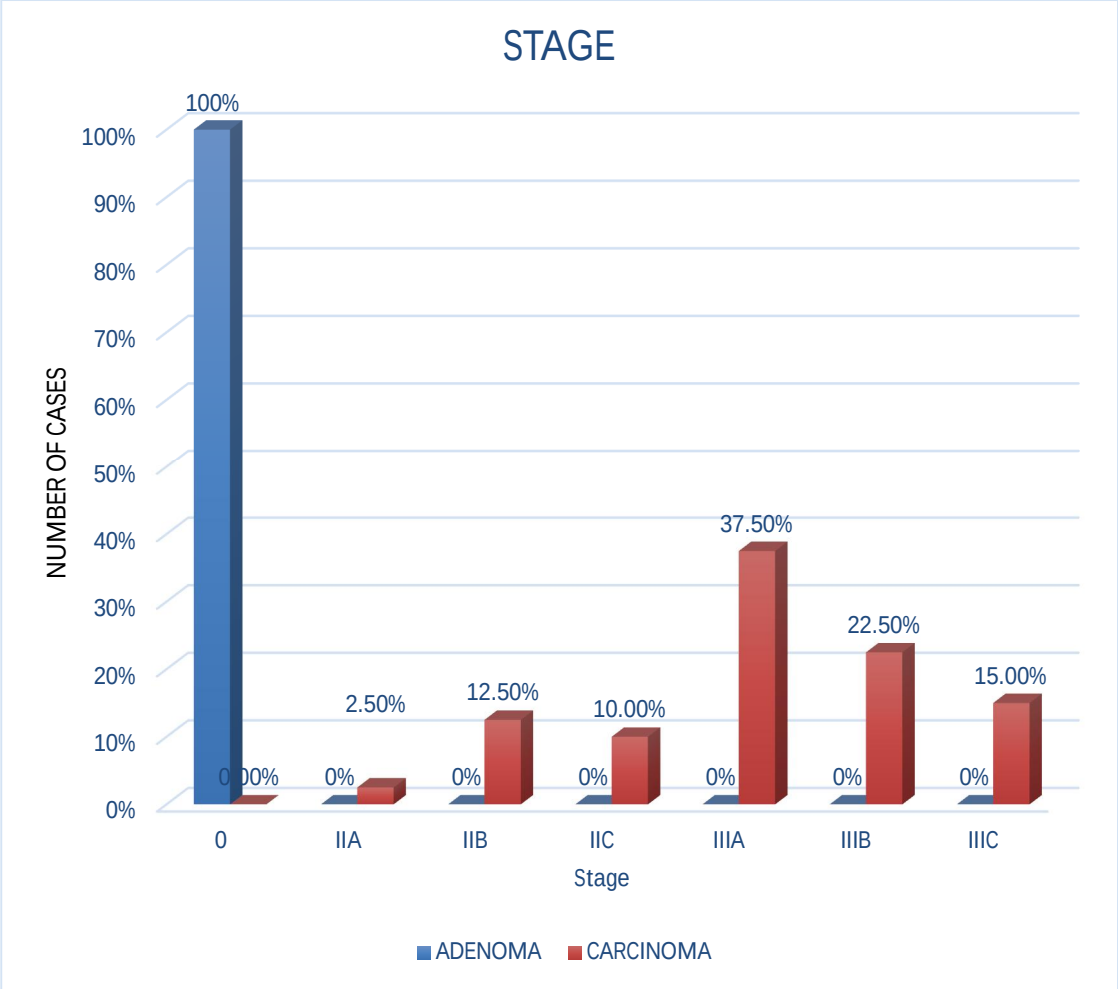
Out of 50 cases ,10 cases of adenoma were taken as stage 0.Outof 40 cases of carcinoma ,according to AJCC staging, 1 case was in stage IIA,5 cases were in stage IIB , 4 cases were in IIC ,15 cases were in stage IIIA, 9 cases were in IIIB, 6 cases were in stage IIIC

Table 7:

STAGE	type of lesion			
	Adenoma		Carcinoma	
	no of cases	%	no of cases	%
0	10	100%	0	0%
IIA	0	0%	1	3%
IIB	0	0%	5	13%
IIC	0	0%	4	10%
IIIA	0	0%	15	38%
IIIB	0	0%	9	23%
IIIC	0	0%	6	15%
TOTAL	100	100%	40	100%

Pearson chi square = 50.00,P value <0.01

Chart **7: Stage wise distribution of cases**



Lymphocytic infiltration among the cases

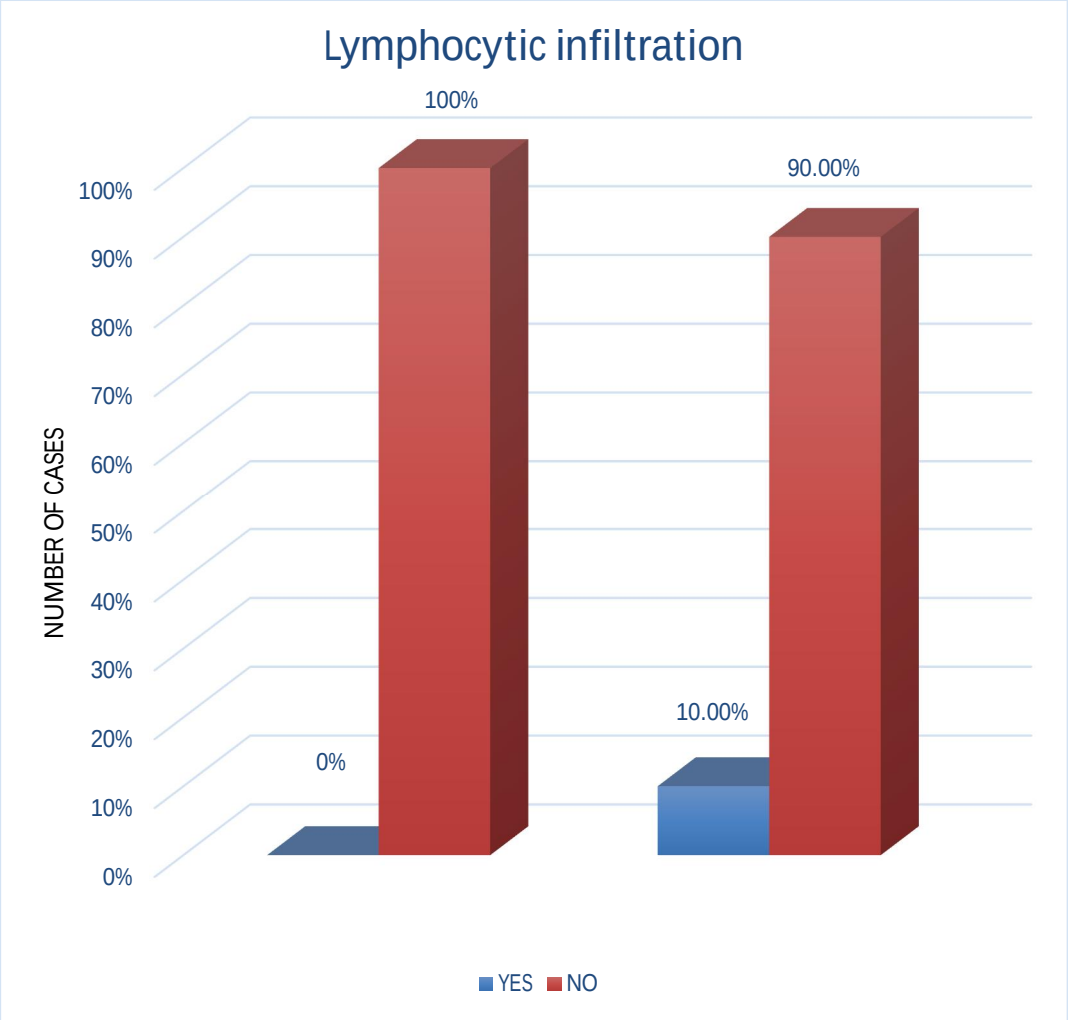
No lymphocytic infiltration is seen in 10 cases of adenoma. In colorectal carcinoma out of 40 cases 4 cases showed lymphocytic infiltration.

Table 8:

LCI	type of lesion			
	Adenoma		Carcinoma	
	no of cases	%	no of cases	%
YES	0	0%	4	10%
NO	10	100%	36	90%
TOTAL	10	100%	40	100%

Pearson chi square = 1.087, P value – 0.297

Chart 8: lymphocytic infiltration among the cases



Lymph node metastasis among the cases

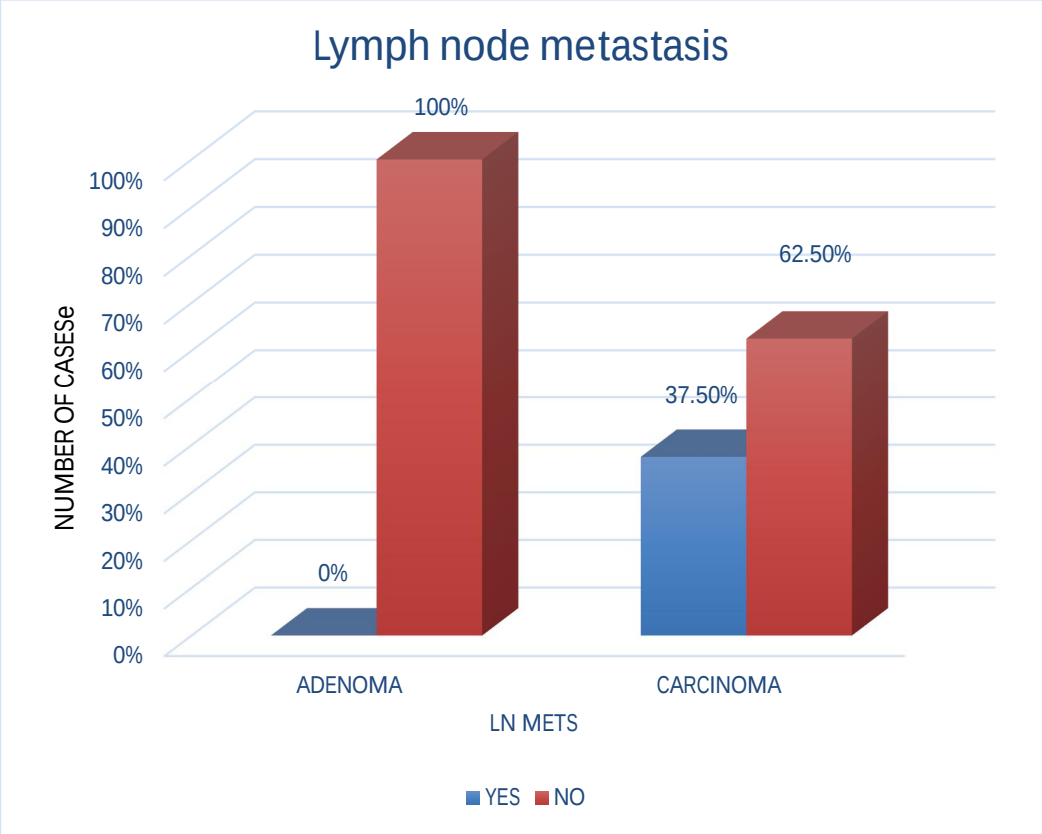
No lymphnode metastasis were seen in 10 cases of adenoma. Out of 40 cases of carcinoma ,15 cases were positive for lymph node metastasis and 25 cases were negative for lymphnode metastasis.

Table 9:

LN METS	type of lesion			
	Adenoma		Carcinoma	
	no of cases	%	no of cases	%
YES	0	0%	15	37.5%
NO	10	100%	25	62.5%
TOTAL	10	100%	40	100%

Pearson chi square =5.357, P value 0.02

Chart 9: Lymph node metastasis distribution among cases



Lympho vascular invasion among the cases

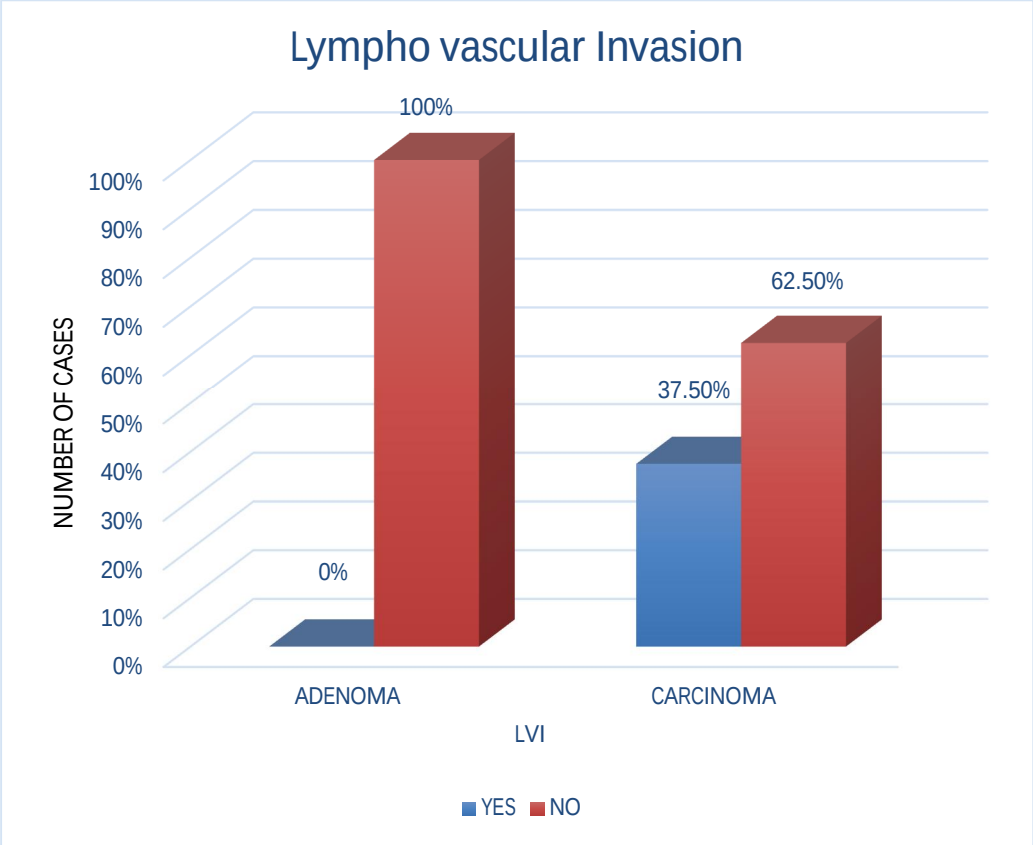
Out of 10 cases of adenoma no case showed lymhovascular invasion out of 40 cases of carcinoma 15 cases showed lymphovascular invasion.

Table 10:

LVI	type of lesion			
	Adenoma		Carcinoma	
	no of cases	%	no of cases	%
YES	0	0%	15	37.5%
NO	10	100%	25	62.5%
TOTAL	10	100%	40	100%

Pearson chi value = 5.357,P value 0.021

Chart 10 : Lymphovascular invasion among the cases



Results of immunohistochemical analysis

Out of 50 cases ,10 cases were adenoma and 40 cases were carcinoma.Positivity is graded based on their intensity separately for membrane,cytoplasmic and nucleus.

0 – negative

1+ circumferential membrane pattern was incomplete

2+ circumferential staining with weak /intermediate intensity

3+ complete strong circumferential positivity

0,1+ were taken as negative ,2+ and 3+ were taken as positive.

Membranous expression of beta catenin among adenomas and carcinomas

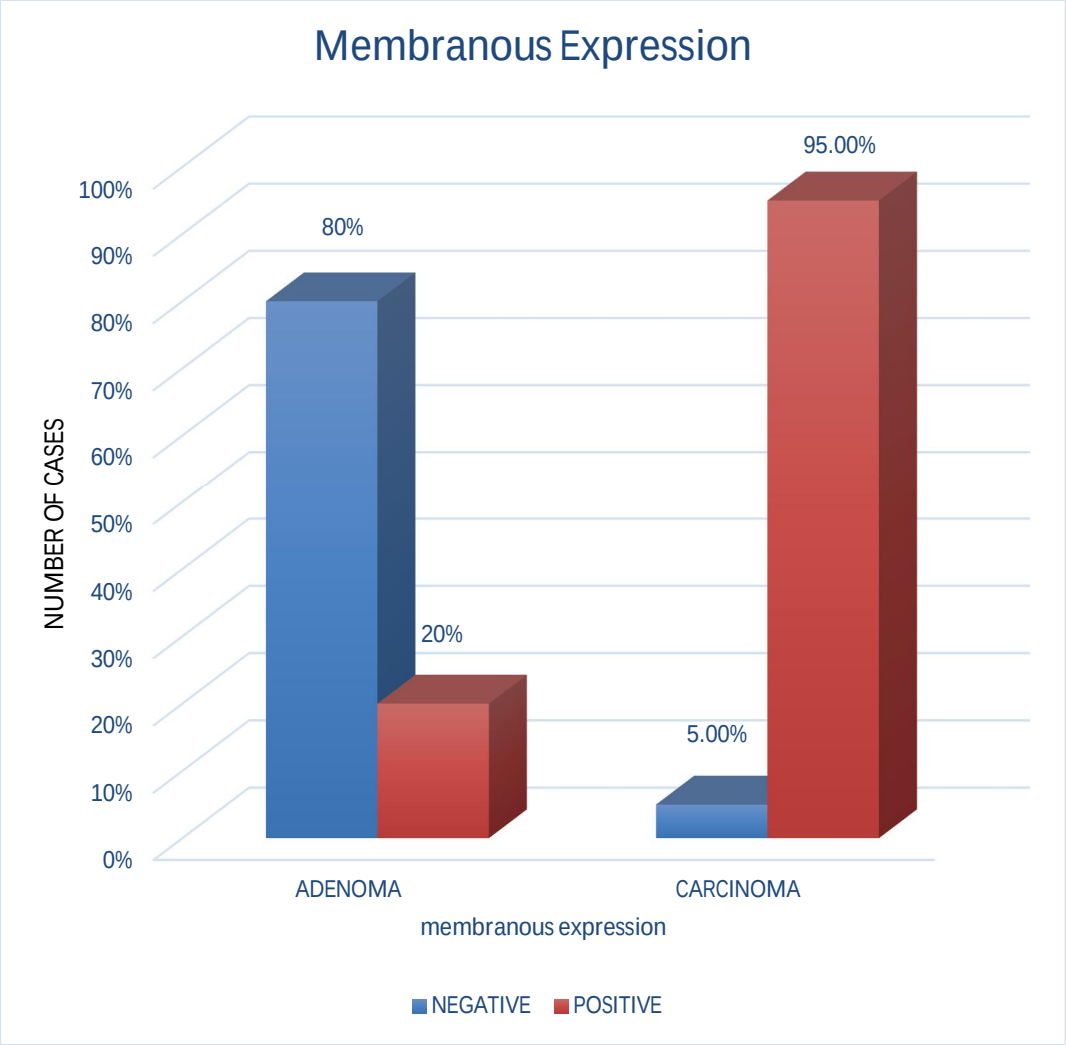
Out of 10 cases of adenoma ,8 cases showed negative for membranous expression and 2 cases showed membranous positivity.Out of 40 cases of carcinoma 2 cases showed negative for membranous expression and 38 cases showed membranous positivity.

Table 11:

Membranous expression of beta catenin	**type of lesion**			
	Adenoma		**Carcinoma**	
	no of cases	**%**	**no of cases**	**%**
NEGATIVE	8	80%	2	5%
POSITIVE	2	20%	38	95%
TOTAL	10	100%	40	100%

Pearson chi square = 28.125,p value < 0.01

Chart 11 : Membranous expression of betacatenin among cases.



Distribution of cytoplasmic beta catein expression among adenoma and carcinoma cases

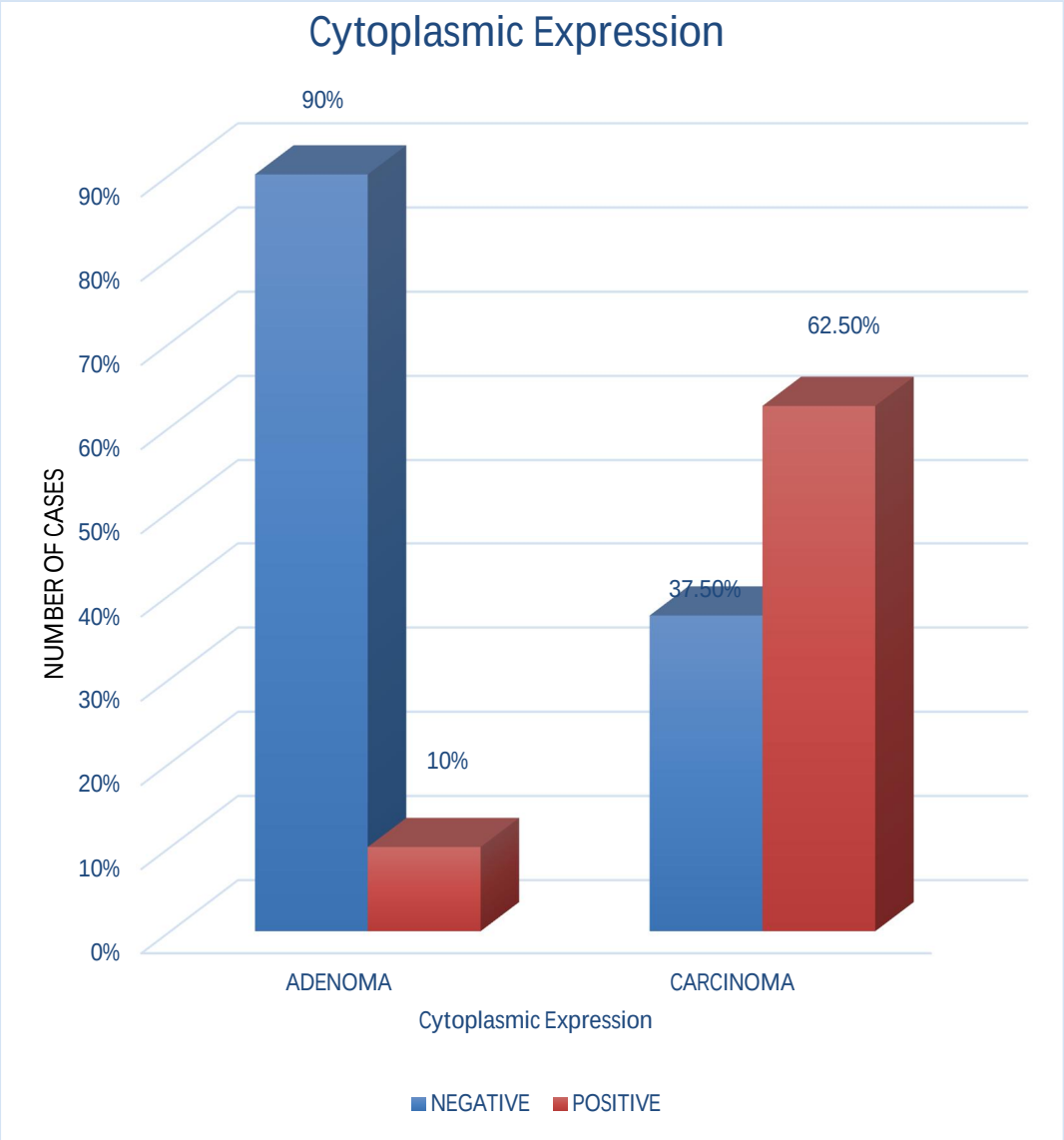
Out of 10 cases of adenoma ,9 showed cytoplasmic negativity ,1 case showed cytoplasmic positivity.Out of 40 cases of carcinoma ,15showed cytoplasmic negativity and 25 cases showed cytoplasmic positivity.

Table 12:

Cytoplasmic expression	**type of lesion**			
	Adenoma		**Carcinoma**	
	no of cases	**%**	**no of cases**	**%**
NEGATIVE	9	90%	15	37.5%
POSITIVE	1	10%	25	62.5%
TOTAL	10	100%	40	100%

Pearson chi square – 8.834,P value – 0.003

Chart 12: cytoplasmic expression of betacatenin among the cases



Distribution of nuclear beta catenin expression among adenoma and carcinoma cases.

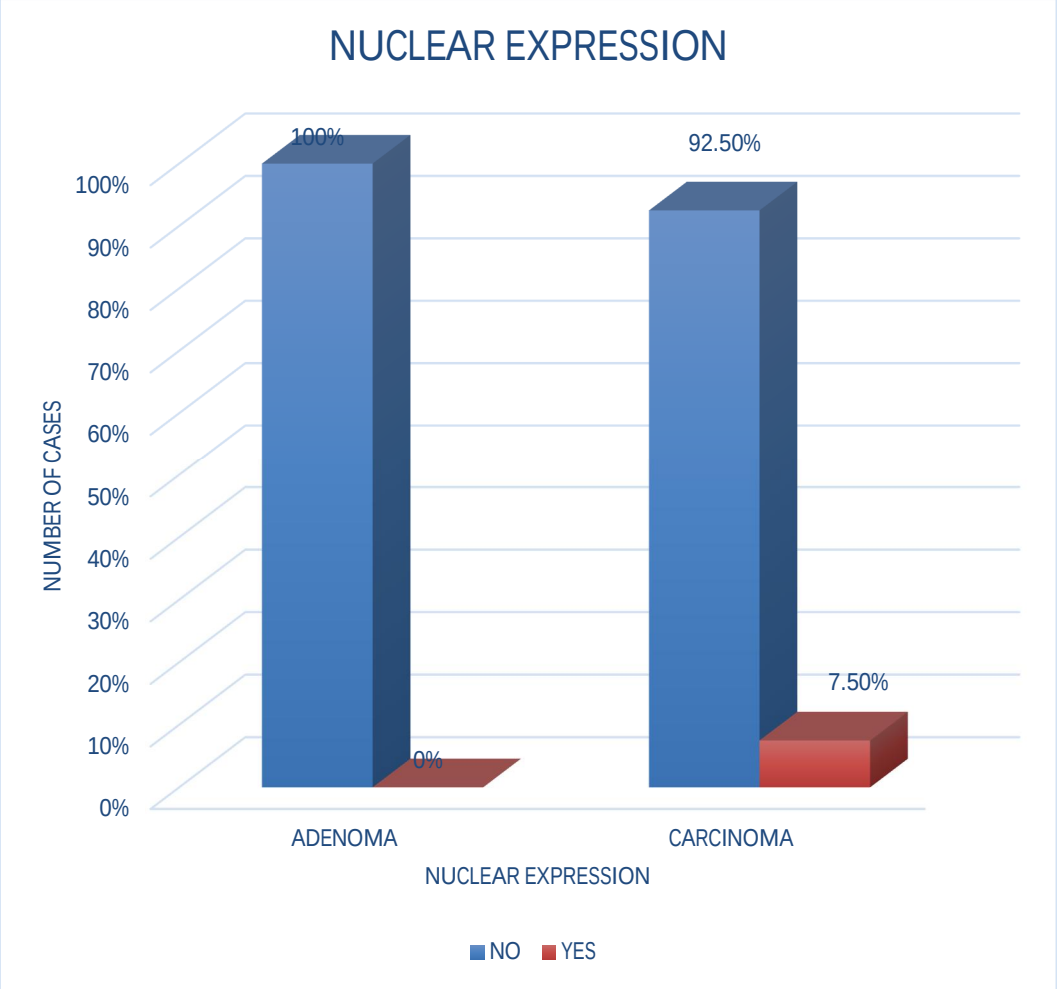
Out of 10 cases of adenoma studied,no case showed nuclear positivity. Out of 40 cases of carcinoma 3 cases showed nuclear positivity.

Table 13:

Nuclear expression of beta-catenin	**type of lesion**			
	Adenoma		**Carcinoma**	
	no of cases	**%**	**no of cases**	**%**
No	10	100%	37	92.5%
Yes	0	0%	3	7.5%
TOTAL	10	100%	40	100%

Pearson chi square = 0.798, p value – 0.372

Chart 13: Nuclear expression of betacatenin among the cases



DISCUSSION

Globally colorectal cancer is a major public health problem. It is the fourth most among the top ten in men and women.

Survival is mainly dependent on the stage of the disease at the time of diagnosis, with a better 5 years survival for the patients diagnosed at the localized stage.

In this study 10 cases of adenoma and 40 cases of carcinoma are taken and is correlated with various clinicopathological parameters.

Comparison of age group of occurrences with other studies,

In this study colorectal adenoma is equally distributed in the age group of 41 –50years,51-60 years and more than 70 years.

Douglas A Corley et al [147] conducted a study in 2013 which showed that the estimated risk of adenoma doubled from age 50-54 years to 70-74years, reaching peak at 70-74years.

Takako Eguchi Nakajima et al [151] conducted a study in 2010, showed that the mean age of occurrence of colorectal adenoma is 66.8years.

In this study the highest incidence of colorectal carcinoma is distributed in the age group of 51 to 60 years.

Troisi et al[152] conducted a study in 223 colorectal carcinoma patients and found that the mean age at diagnosis is 60 years .

Robin P Burshey et al [153]conducted a study in 168 colorectal carcinoma patients and conducted that the median age at diagnosis was 72 years.

Chu Kc et al[154] **and Gendi et al** [155] conducted studies in 108 and 159 colorectal carcinoma patients and found that the median age of diagnosis was 58 and 55years.

Comparison of location of adenoma and carcinoma with other studies.

In this study both adenoma and carcinoma showed left sided preponderance. Study by **Takako Eguchi Nakajima et al**[156]in the year 2010, showed left sided preponderance for adenoma and carcinoma.

In study conducted by **Kazem et al**[157] and **Scott et al**[158],left sided tumors constituted about 56.7%,69.2% and right sided constituted about 43% and 308% respectively.

Comparison of histological subtype of colorectal carcinoma with another study

Histological type	**Kazem et al**	**Current study**
Infiltrating adenocarcinoma	**86.7%**	**85%**
Mucinous adenocarcinoma	**10%**	**12.5%**
Infiltrating adenocarcinoma with mucinous differentiation	**-**	**2.5%**

In the study conducted by **Kazem et al** in 323 colorectal carcinoma ,the most common microscopic subtype was infiltrating adenocarcinoma which constituted about 86.7% followed by mucinous carcinoma which was about 10%.

The main histological subtype of colorectal carcinoma in the current study is infiltrating adenocarcinoma which is in concurrence with the study of **Kazem et al**.

Comparison of expression of beta catenin in colorectal adenoma and carcinoma with other studies.

Complete membrane positivity is seen in 20% of adenoma and 95% of carcinoma.In the study conducted by **M.Kobayashi et al**[159] in 75 patients showed all cases of adenoma showed preserved type of cell membrane immune

staining pattern and reduced expression in colorectal carcinoma. In the current study cytoplasmic positivity is seed in 10% of adenoma and 62.5% of carcinoma. Inthe study conducted by **M.Kobayashi et al** in 75 patients showed cytoplasmic staining intensity is higher in adenoma than carcinoma**.**

In the current study no case of adenoma showed positivity for nuclear beta catenin, were as 3 out of 40 cases showed nuclear positivity. In this study conducted by **M.Kobayashi et al** in 75 patients showed no case of adenoma showed diffuse nuclear positivity.4% of intramucosal and 17% of invasive cancers showed nuclear positivity.

SUMMARY

50 colectomy specimens from the year 2016-2018 were taken. Out of which 10 were adenomas and 40 were carcinomas. The mean age of occurrence of colorectal adenoma is 57.7 years

Colorectal carcinoma is 48.8 years. Youngest age of occurrence of colorectal adenoma is 42years, colorectal carcinoma is 18years.Oldest age of presentation of colorectal adenoma is 80 years and colorectal carcinoma is 73 years.

There is female preponderance in adenoma and male preponderance in carcinoma. There is left sided preponderance in both adenoma and carcinoma.70% of adenomas and 62.5 % of carcinomas are left sided. Commonest gross appearance is ulceroproliferative growth in adenomas and Carcinomas.

Most common histological subtype is infiltrating adenocarcinoma, which constitutes 85%among the carcinomas.Commonest grade is well differentiated, which constitutes 67.5% of all carcinomas.

37% of cases belong to AJCC classification stage IIIa.10% of carcinomas showed lymphocytic infiltration.Resected margins are free in all 50 cases.15 cases ie 37.5% of cases showed lymph node metastasis.

37.5% of cases showed lymphovascular invasion. Statically significant association P value less than 0.05 is present in membranous and cytoplasmic expression of adenoma and carcinoma.20% of adenoma and 95% of carcinomas showed membranous positivity.10% of adenoma and 62.5% of carcinoma showed cytoplasmic positivity. There is no significant association in nuclear expression of beta catenin between adenoma and carcinoma.

CONCLUSION

Among 50 cases studied at kilpauk medical college from 2016 -2018, mean age of presentation in colorectal adenoma is 57.7 years and colorectal carcinoma is 48.8 years . Colorectal adenoma showed female preponderance,colorectal carcinoma showed male preponderance. Both adenoma and carcinoma showed left sided preponderance. Well differentiated Infiltrating adenocarcinoma being the predominant histopathological subtype. Immunohistochemical analysis was performed to assess the expression of beta catenin in colorectal adenoma and carcinoma Significant association is present in membranous and cytoplasmic expression of beta catenin, which is higher in carcinoma when compared with adenoma.Out of 50 cases only 3 cases of carcinoma showed nuclear positiv**ity. This study throws light on the need for targeted therapy against beta catenin which is overexpressed in carcinomas compared with adenoma. Nuclear expression of beta catenin has an impact on prognostic features such as invasiveness and metastatic potential.Beta** catenin is an important oncoprotein in colorectal carcinoma and its nuclear expression has definitive value as a prognostic factors such as invasiness and metastastic potential .

FIG 9 : COLORECTAL CARCINOMA – GROSS

Fig 10 : COLORECTAL ADENOCARCINOMA

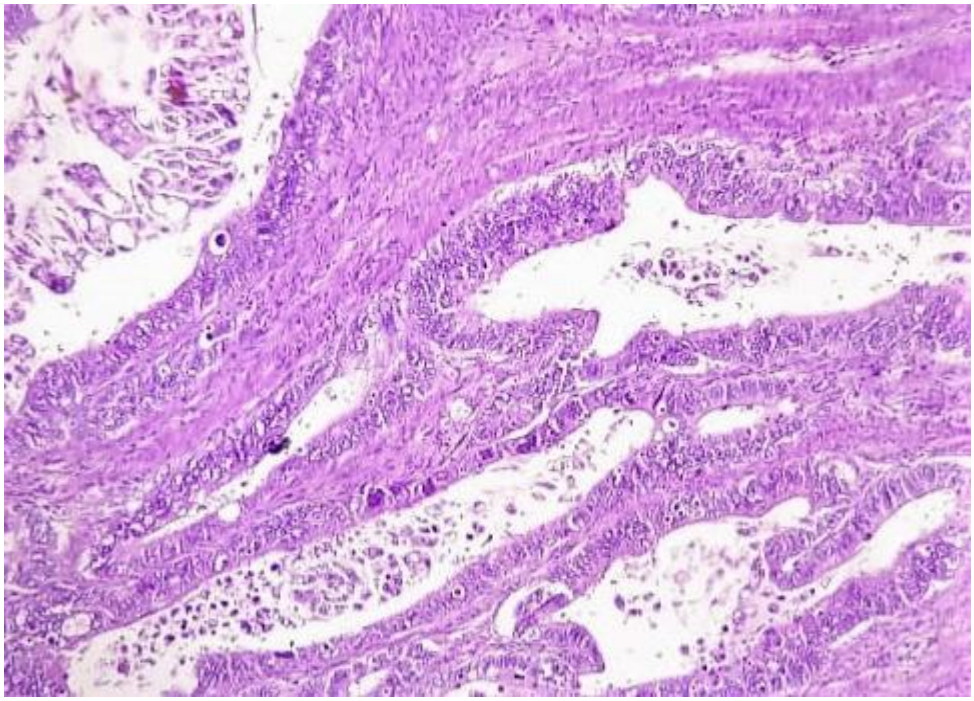


Fig 11 : TUBULOVILLOUS ADENOMA

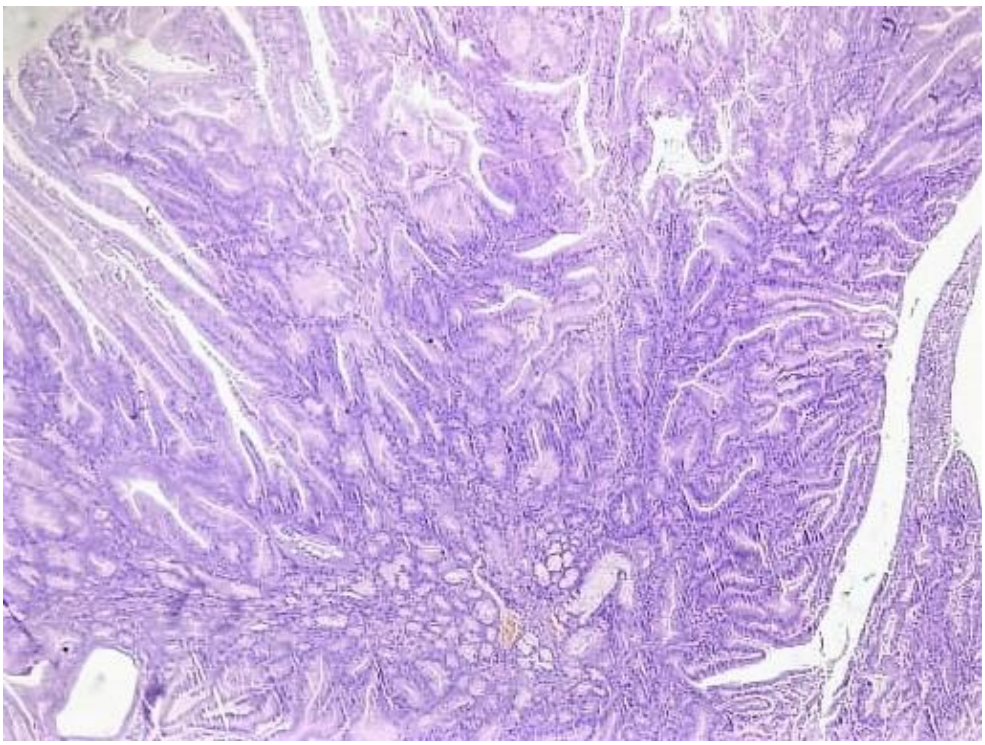


Fig 12 :BETACATENIN IN NORMAL INTESTINAL MUCOSA

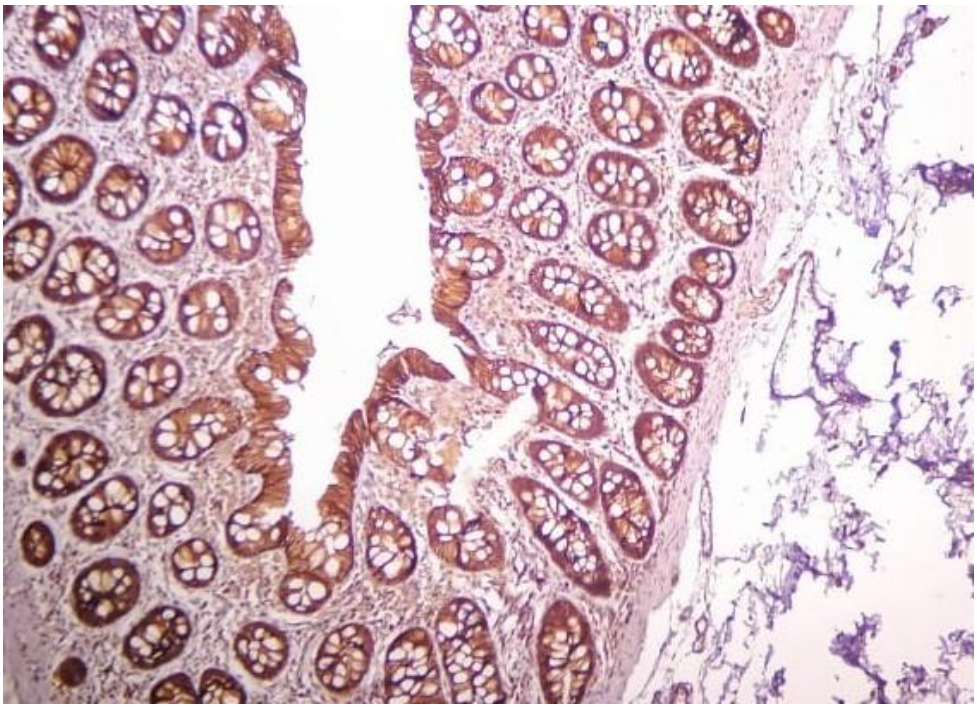


Fig 13 : BETACATENIN IN ADENOMA

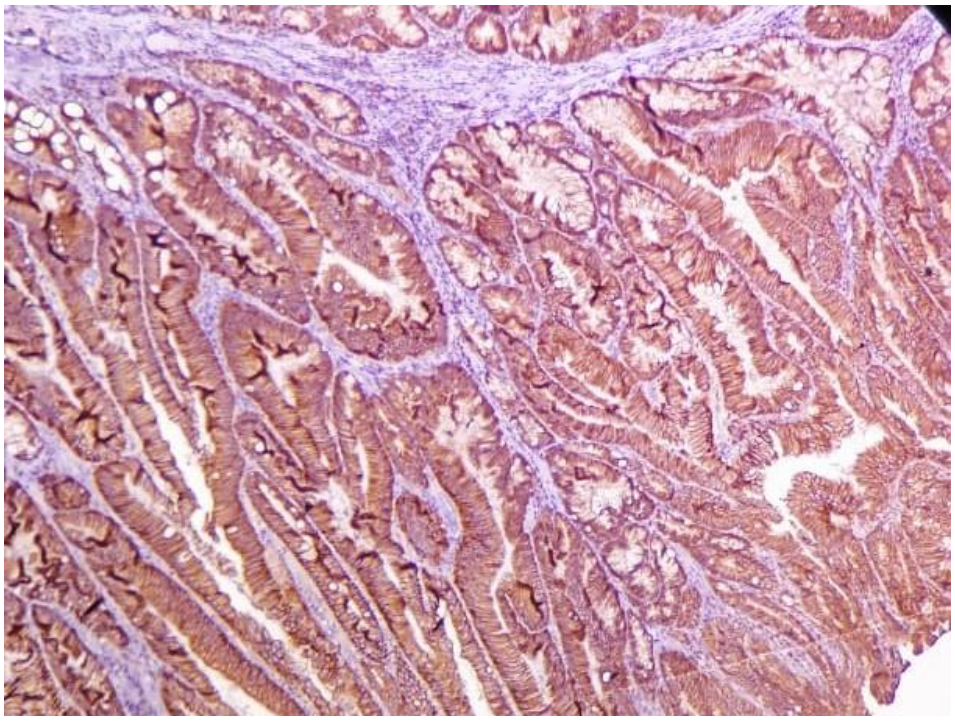


Fig 14 :BETACATENIN IN COLORECTAL CARCINOMA-MEMBRANOUS EXPRESSION

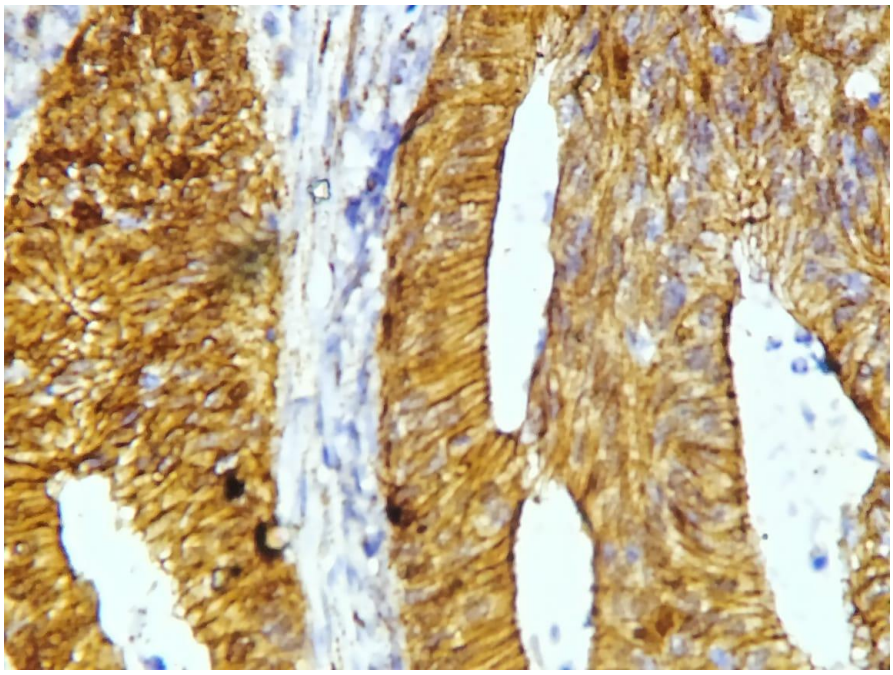
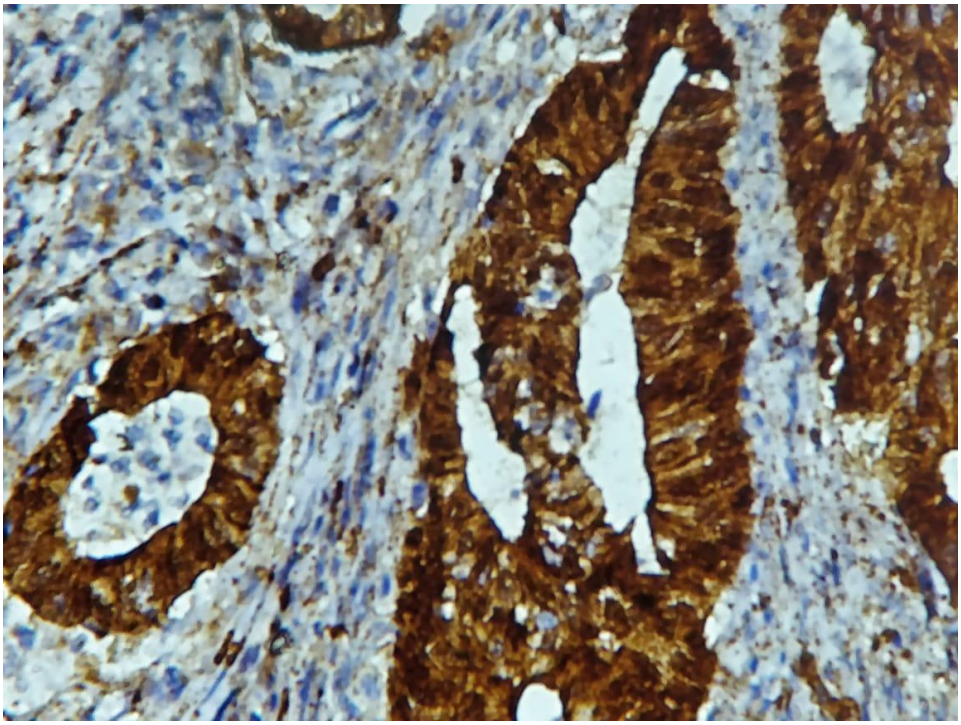


Fig 15 : NUCLEAR BETACATENIN EXPRESSION



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ANNEXURE I

PROFORMA

Case number : **Name :**

HPE number : **Age:**

IP number : **Sex :**

Clinical features :

Clinical diagnosis :

Previous HPE report:

Nature of specimen :

Tumour site :

MICROSCOPY:

Histological grade :

IMMUNOHISTOCHEMISTRY

Beta catenin scoring.

ANNEXURE II

WHO CLASSIFICATION OF TUMORS COLON AND RECTUM

Epithelial tumors :

Premalignant lesions

- **Adenoma**
- **Tubular**
- **Villous**
- **Tubulovillous**
- **Dysplasia – low grade**
- **Dysplasia – high grade**

Serrated lesions

- **Hyperplastic polyp**
- **Sessile serrated adenoma / polyp**
- **Traditional serrated adenoma**

Hamartomas

- **Cowden associated polyp**
- **Juvenile polyp**
- **Peutz – Jeghers polyp**

Carcinomas

- **Adenocarcinoma**
    1. **Cribriform comedo type adenocarcinoma**
    2. **Medullary adenocarcinoma**

3. **Micropapillary carcinoma**
4. **Mucinous adenocarcinoma**
5. **Serrated adenocarcinoma**
6. **Signet ring cell carcinoma**

- **Adenosquamous carcinoma**
- **Spindle cell carcinoma**
- **Squamous cell carcinoma**
- **Undifferentiated carcinoma**

Neuroendocrine neoplasms

- **Neuroendocrine tumor (NET)**

  NET G1 (CARCINOID)

  NET G2

- **Neuroendocrine carcinoma**

Large cell NEC

Small cell NEC

- **Mixed adenoneuroendocrine carcinoma**
- **EC cell ,serotonin producing NET**
- **L cell, Glucagon – like pepite producing NET**

Mesenchymal tumors :

- **Leiomyoma**
- **Lipoma**
- **Angiosarcoma**
- **Gastrointestinal stromal tumor**
- **Kaposi sarcoma**
- **Leiomyosarcoma**

Lymphomas

Secondary tumors

ANNEXURE III

American Joint Committee on Cancer (AJCC) TNM Classification of colorectal carcinoma

Tumor

- Tis in situ dysplasia or intramucosal carcinoma
- T1 tumor invades submucosa
- T2 tumor invades but not through,muscularis propria
- T3 tumor invades through muscularis propria
- T3a invasion less than 0.1 cm beyond muscularis propria
- T3b invasion 0.1 to 0.5cm beyond muscularis propria
- T3c invasion 0.5 to 1.5 cm beyond muscularis propria
- T3d invasion more than 1.5 cm beyond muscularis propria
- T4 tumor penetrates visceral peritoneum or invades adjacent organs
- T4a Penetration into visceral peritoneum
- T4b invasion in to other organs or structures
- Regional lymph nodes
- Nx lymph node cannot be assesed
- N0 no regional lymph node metastasis
- N1 metastasis in one to three regional lymph nodes
- N1a metastasis in one regional lymphnode
- N1b metastasis in one two or three regional lymph nodes
- N1c tumor deposits in the subserosa ,mesentry, or non peritonealised
    - Pericolic or perirectal tissue without regional nodal metastasis
- N2 metastasis in four or more regional lymphnodes
- N2a metastasis in four to six regional lymphnodes
- N2b metastasis in seven or more regional lymph nodes
- Distant metastasis
- Mx distant metastasis cannot be assessed
- M0 no distant metastasis
- M1 distant metastasis
- M1a metastasis confined to one organ or site
- M1b metastasis in more than one organ / site or the peritoneum

Colorectal carcinoma staging

Stage	T	N	M
I	T1,T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1-T2	N1/N1c	M0
	T1	N2a	M0
III B	T3, T4a	N2a	M0
	T2, T3	N2a	M0
	T1,T2	N2b	M0
IIIC	T4a	N2a	M0
	T3,T4a	N2b	M0
	T4b	N1,N2	M0
IVA	Any T	Any N	M1a
IV B	Any T	Any N	M1b

KEY TO MASTER CHART

SEX

1-MALE

2-FEMALE

P/D-PROCEDURE DONE

1- RIGHT HEMICOLECTOMY

2- LEFT HEMICOLECTOMY

3- ABDOMINOPERINEAL RESECTION

4- ANTERIOR RESECTION

5- SIGMOID COLON RESECTION

LOCATION

1-RIGHT

2-LEFT

GROSS APPEARANCE

1-ULCEROPROLIFERATIVE

2-ULCERATIVE

3-ULCERONODULAR

4-CIRCUMFERENTIAL

5-STRICTURE

6-POLYPOIDAL

HPE-HISTOPATHOLOGICAL EXAMINATION

1-INFILTRATING ADENOCARCINOMA

2-INFILTRATING ADENOCARCINOMA WITH MUCINOUS DIFFERENTIATION

3-MUCINOUS CARCINOMA

GRADE

0 -ADENOMA

1-WELLDIFFERENTIATED

2-MODERATELYDIFFERENTIATED

3-POORLYDIFFERENTIATED

LN METS-LYMPH NODE INVOLVEMENT

P -PRESENT

N-NEGTIVE

LVI-LYMPHATIC INVASION

P -PRESENT

N-NEGTIVE

LCI-LYMPHOCYTIC INFILTRATION

P -PRESENT

N-NEGTIVE

STAGING

1-STAGE 0

2-STAGE I

3-STAGE IIA

4- STAGE IIB

5- STAGE IIC

6- STAGE IIIA

7-STAGE IIIB

8-STAGE IIIC

9-STAGE IVA

10.STAGE IVB

IHC SCORE

0- NEGATIVE

1- 1+ INCOMPLETE WEAK MEMBRANE POSTIVE

2- 2+ COMPLETE WEAK MEMBRANE POSITIVE

3- 3+COMPLETE INTENSE POSITIVE

M – MEMBRANOUS EXPRESSION

C- CYTOPLASMIC EXPRESSION

N – NUCLEAR EXPRESSION

MASTER CHART

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
1	334/15	53	m	2	6*5*5	2	1	1	1	IIIa	N	N	N	-	M-2+ C-2+ N-neg
2	933/15	23	f	2	2*2*2	2	1	1	3	IIIb	P	N	P	-	M-2+ C-2+ N -neg
3	976/15	35	m	1	5*2*2	1	6	1	2	IIIb	P	N	P	-	M-2+ C-2+ N-neg
4	1149/15	30	m	2	2*2*2	5	5	1	1	IIIb	P	P	P	-	M-2+ C-2+ N-neg
5	1182/15	79	m	2	4*3*2	4	1	1	2	IIIa	N	N	N	-	M-2+ C-2+ N-neg
6	1453/15	66	m	1	7*6*5	1	1	3	1	IIIa	N	N	N	-	M-2+ C-2+ N-neg

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
7	1533/15	51	f	1	6*6*5	1	1	2	1	IIIa	N	N	N	-	M-2+ C-2+ N-neg
8	1823/15	46	m	1	2*2*2	1	6	0	0	0	N	N	N	M-1+ C-1+ N-neg	
9	2206/15	22	m	1	5*3*2	1	2	3	1	IIIa	N	N	N		M-2+ C-2+ N-neg
10	2403/15	45	f	2	7*4*4	5	1	1	1	IIa	N	N	N		M-1+ C-1+ N-neg
11	2500/15	35	m	1	4*2*2	1	1	1	2	IIIa	N	N	N		M-2+ C-1+ N-neg
12	2596/15	60	f	1	3*3*3	1	3	1	3	IIIa	N	N	N		M-2+

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
															C-1+ N-neg
13	70/16	51	m	1	4*2*1	1	1	1	2	IIIb	P	N	P		M-3+ C-2+ N-neg
14	370/16	75	m	2	6*6*3	2	3	1	1	IIIb	N	N	N		M-3+ C-2+ N-neg
15	492/16	61	m	2	4*3*3	2	1	1	2	IIIb	p	p	p		M-3+ C-3+ N-neg
16	545/16	67	f	2	4*4*3	2	4	0	0	0	N	N	N	M-1+ C-1+ N-neg	
17	624/16	25	f	2	4*3*2	2	1	1	1	IIIa	P	N	P		M-2+ C-1+ N-neg

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
18	671/16	45	m	1	6*5*5	1	1	3	1	IIIb	P	N	P		M-3+ C-3+ N-+
19	1470/16	52	f	2	2*2*2	2	1	1	2	IIIa	N	N	P		M-2+ C-1+ N-neg
20	2110/16	40	f	2	4*3*2	2	2	3	1	IIIb	p	p	p		M-2+ C-2+ N-neg
21	2714/16	50	f	1	4*3*3	1	6	1	1	IIb	P	N	P		M-2+ C-2+ N-neg
22	2778/16	50	f	1	3*3*3	1	1	1	2	IIIb	P	N	P		M-3+ C-3+ N-+
23	105/17	73	f	2	4*3*3	2	1	0	0	0	N	N	N	M-1+ C-1+	

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
														N-neg	
24	116/17	75	m	2	4*2*2	2	1	1	2	IIa	N	N	N		M-1+ C-0+ N-neg
25	185/17	72	m	2	5*5*3	2	4	0	0	0	N	N	N	M-1+ C-1+ N-neg	
26	201/17	33	m	2	10*6*4	2	3	3	2	IIIa	N	N	N		M-2+ C-1+ N-neg
27	274/17	58	f	1	4*2*2	1	1	1	1	IIa	N	N	N		M-2+ C-2+ N-neg
28	318/17	18	m	2	7*4*2	2	1	1	1	IIa	N	N	N		M-2+ C-2+ N-neg
29	817/17	48	m	2	8*4*4	2	2	1	1	IIb	N	N	N		M-2+ C-2+

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
															N-neg
30	973/17	42	f	2	4*3*3	2	1	0	0	0	N	N	N	M-2+ C-2+ N-neg	
31	1296/17	76	m	2	5*4*4	2	1	1	1	IIIb	P	N	N		M-3+ C-3+ N-neg
32	1339/17	52	m	2	4*3*3	2	1	1	1	IIa	N	N	N		M-2+ C-1+ N-neg
33	1672/17	42	f	2	7*5*5	2	1	1	1	IIa	N	N	N		M-2+ C-1+ N-neg
34	1742/17	78	m	2	3*2*2	2	1	1	1	IIb	N	N	N		M-2+ C-1+ N-neg

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
35	1750/17	55	f	2	6*5*5	2	1	1	1	IIIa	N	N	N		M-2+ C-1+ N-neg
36	2056/17	40	m	1	3*2*2	1	4	1	1	IIIa	N	N	N		M-3+ C-2+ N-neg
37	2216/17	40	f	1	12*11*10	1	1	1	1	IIIb	P	N	P		M-3+ C-3+ N-+
38	2344/17	80	F	2	4*3*3	2	1	0	0	0	N	N	N	M-1+ C-1+ N-neg	
39	2632/17	55	F	2	6*4*4	2	1	0	0	0	N	N	N	M-1+ C-1+ N-neg	
40	2915/17	59	M	2	6*3*3	2	1	1	1	IIIa	P	N	P		M-2+ C-2+ N-neg

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
41	**49/18**	**53**	**m**	**1**	**3.5*3*2**	**1**	**1**	**1**	**1**	**IIIa**	**N**	**N**	**N**		**M-2+ C-1+ N-neg**
42	**144/18**	**38**	**f**	**2**	**7*4*4**	**2**	**2**	**1**	**1**	**IIIa**	**N**	**N**	**N**		**M-2+ C-1+ N- neg**
43	**356/18**	**23**	**f**	**2**	**1*1*1**	**2**	**2**	**1**	**1**	**IIa**	**N**	**N**	**N**		**M-2+ C-1+ N-neg**
44	**458/18**	**60**	**f**	**1**	**5*5*2**	**1**	**1**	**0**	**0**	**0**	**N**	**N**	**N**	**M-1+ C-2+ N-neg**	
45	**826/18**	**42**	**f**	**1**	**6*5*5**	**1**	**1**	**0**	**0**	**0**	**N**	**N**	**N**	**M-2+ C-2+ N-neg**	
46	**1480/18**	**33**	**m**	**2**	**6*1.5*1**	**2**	**1**	**1**	**1**	**IIIa**	**N**	**N**	**N**		**M-3+**

s.no	HPE .no	Age	Sex	Location	Size (cm)	P/D	Gross	HPE	Grade	Stage	LN METS	LCI	LVI	B-catenin in adenoma	β-catenin in carcinoma
															C-2+ N-neg
47	1628/18	58	M	1	5*3*3	1	1	1	1	IIIa	P	N	P		M-3+ C-3+ N-+
48	1256/18	60	f	2	3*3*3	2	1	0	0	0	N	N	N	M-1+ C-2+ N-neg	
49	1360/18	55	f	2	3*2*1	2	1	1	2	IIa	N	N	N		M-2+ C-1+ N-neg
50	1480/18	70	m	2	4*3*3	2	1	1	2	IIIa	N	N	N		M-2+ C-1+ N-neg